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Periodontal Disease as a Specific, albeit Chronic, Infection: Diagnosis and Treatment

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THE CLINICAL CONDITION

Periodontal disease(s) refers to the inflammatory processes that occur in the tissues surrounding the teeth in response to bacterial accumulations (dental plaque) on the teeth. Rarely do these bacterial accumulations cause overt infections, but the inflammatory response(s) which they elicit in the gingival tissue is ultimately responsible for a progressive loss of collagen attachment of the tooth to the underlying alveolar (jaw) bone, which, if unchecked, can cause the tooth to loosen and then to be lost. The resulting crevice between the tooth surface and the approximating epithelial surface is called the periodontal pocket. This pocket can extend from 4 to 12 mm and can harbor, depending on its depth and extent, from 10^7 to almost 10^9 bacterial cells (281). The gingival bleeding and attachment loss associated with this process is usually painless and is ignored by the individual. Often the first time that the individual is aware of the problem is when the dentist informs him or her of the presence of pockets measuring more than 4 mm in depth. For example, the individual in Fig. 1 came to the dental clinic seeking replacement of his missing front tooth and had to be told that he had advanced periodontal disease with many deep pockets, ≥5 mm in depth. This symptomless nature of periodontal disease is one of its defining characteristics.

The prevalence of periodontal disease increases with age (36, 87, 88, 145, 217, 219) and as more people are living longer and retaining more teeth, the number of people developing periodontal disease will increase in the next decades. About 50% of the adult population has gingivitis (gingival inflammation without any bone loss about teeth and no pockets deeper than 3 mm) around three or four teeth at any given time, and 30% have periodontitis as defined by the presence of three or more teeth with pockets of ≥4 mm (9, 217). Between 5 and 15% of those with periodontitis have advanced forms with pockets of ≥6 mm (219). Another 3 to 4% of individuals will develop an aggressive form of periodontal disease, known as early onset periodontitis (EOP), between the ages of 14 and 35 years. Any medical condition that affects host antibacterial defense mechanisms, such as human immunodeficiency virus infection HIV (328), diabetes (219, 264), and neutrophil dis-

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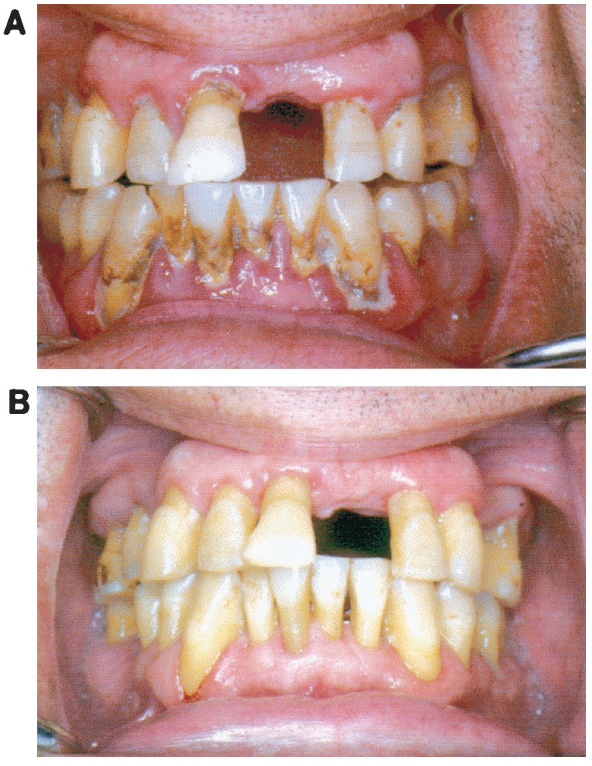

FIG. 1. Patient with advanced adult periodontitis. (A) Prior to treatment. The patient came to the clinic seeking replacement for missing tooth. He was not aware of having a periodontal problem, despite gingival bleeding and massive amounts of supragingival plaque and calculus about the teeth. Note the whitish material at junction where the teeth contact the gingival tissue, especially about the canine tooth on the lower right. (B) After the plaque and calculus have been removed. There has been considerable bone loss about certain teeth, which accounts for the exposed roots.

orders (312), will predispose the individual to periodontal disease.

These prevalences suggest that about two million Americans younger than 35 years and another four million older than 35 years may have a form of periodontal disease that requires professional intervention. Traditional treatments reflect the premise that periodontal disease is due to the nonspecific overgrowth of any and all bacteria on the tooth surfaces and that the magnitude of the bacterial overgrowth on the teeth can be controlled by professional cleaning of the teeth at regular intervals. If these accumulations are not removed, various bacterial by-products and their cellular components such as lipopolysaccharides (LPS), antigens, or enzymes can provoke an inflammatory response in the gingival tissue. Undisturbed plaques often become calcified, forming dental calculus or tartar, which, if formed below the gingival margin, is often difficult to remove from the root surfaces without some form of surgical access.

The patient whose mouth is shown in Fig. 1A did not practice good oral hygiene, nor did he have a dentist clean his teeth at regular intervals, and so he accumulated massive amounts of plaque and calculus on his teeth. When his teeth were "cleaned," the gingival inflammation decreased (Fig. 1B). Because the residual depths of many pockets were ≥5 mm, large numbers of bacteria could still accumulate and not be accessible to normal tooth-cleaning procedures. Therefore, periodontal surgery was performed to reduce the pocket depths to 1 to 2 mm, so that the patient would be able, by good brushing techniques, to keep the bacterial load reduced, thereby preventing reoccurrence of inflammation and attachment loss. Professional toothcleanings were recommended every 3 to 4 months for the rest of his life. At no time during the patient's treatment was there a need for a bacteriological diagnosis of the type of bacteria present in this nonspecific overgrowth.

This type of periodontal treatment is the standard of care in periodontal treatment and is based on the premise that if the bacterial overgrowth in dental plaque can be continuously suppressed by mechanical debridement, gingival and periodontal health will be maintained. It is the basis of the "plaque control" programs of organized dentistry and dentifrice manufacturers; as a public health effort, this approach has been very successful. One of the findings of the population-based dental surveys conducted in the last 20 years by the National Institute of Cranio-Facial and Dental Research has been the good overall periodontal health of U.S. citizens (217). However, as noted above, about 2 to 6 million people could require professional treatment, which would include "pocket elimination" surgery so as to gain access to plaque- and calculus-laden root surfaces. Since the average cost for full mouth periodontal surgery is about $4,000 to $5,000, and if 300,000 people (about 10%) actually received treatment, the projected cost could be more than one billion dollars. This would be an overwhelming liability for insurance companies and health care plans to cover. Accordingly very few, if any, dental insurance plans include the full cost of periodontal surgery, and it is not covered by Medicare or Medicaid. This out-of-pocket cost to the individual, plus patient concerns over the surgical procedures themselves (29, 187, 190), would discourage some individuals from seeking treatment.

Such chronic, asymptomatic periodontal infections may go unnoticed by the individual, as evidenced by the person whose mouth is shown in Fig. 1. Recent findings have indicated that chronic infections could serve as a source of inflammatory mediators, LPS, and other bioactive molecules that might contribute to the development of cardiovascular disease (56, 57, 188). Moderate increases in the level of C-reactive protein (CRP) in serum were predictive of new heart episodes in apparently healthy men (234). Others have shown that edentulism (all teeth missing) and periodontal disease are associated with elevated CRP levels in serum after controlling for established risk factors (267). In the case of periodontal disease, the magnitude of the association with CRP levels was comparable to that of chronic bronchitis and cigarette smoking and was strongest for individuals with no medical risk factors, i.e., healthy individuals (267).

Another mechanism by which periodontal bacteria could contribute to cardiovascular pathology relates to the antigenic similarity of certain bacterial proteins with host proteins. For example, subgingival plaque bacteria may share antigenic determinants, such as heat shock proteins (hsp), with host cells. Many host tissues, including the endothelial lining of blood vessels, produce hsp60 as they respond to certain stressors like high blood pressure and LPS. Xu, Wick, and coworkers have postulated that an autoimmune mechanism in which the host responds to foreign hsp60, such as bacterial hsp, could be

important in the development of an atheroma (focal deposit of acellular, mainly lipid-containing material on the endothelial lining of arteries) (322, 335). The sera and inflamed gingival tissues of periodontal patients exhibited a positive antibody response to both the hsp produced by *Porphyromonas gingivalis*, i.e., GroEL hsp60, and to human hsp60 (291). Antibodies to GroEL hsp60 cross-reacted with human hsp60 and vice versa, suggesting that molecular mimicry between molecules of the bacteria and host could play a role in periodontal as well as humoral immune mechanisms. For example, antibodies against the hsps of *P. gingivalis* could react with human hsps exposed on the endothelium and produce cellular damage.

At least 14 of 17 studies of different design and rigor have provided statistical evidence for an association between periodontal disease and cardiovascular disease (27, 160, 188), raising the possibility that periodontal disease is a risk factor for cardiovascular disease. If so, periodontal disease, because it is both preventable and treatable, becomes a modifiable risk factor for cardiovascular disease. However, if periodontal disease is primarily due to the overgrowth of bacteria in the dental plaque, all individuals would need preventive treatment, since all individuals form dental plaque. If the focus were on the treatment of existing periodontal disease, the prospects for control would still not be good due to the projected costs of maintaining, with professional supervision, a clean mouth for a lifetime. Even if this cost could be met, the current standard of care, i.e., the debridement and surgical approach, fails in about 15 to 20% of treated individuals, the so-called refractory patients (111, 189, 190).

This scenario assumes that periodontal disease is the host inflammatory response to the bacterial accumulations on the tooth surface and that the types of bacteria present in the overgrowth is not important. But what would be the treatment options if the bacterial overgrowth always, or usually, resulted in the selection of a limited number of bacterial types in the plaque? Could this convergence on a common bacterial profile in disease-associated plaques be considered an infection, albeit a chronic one? If this were the actual situation, there would be a need to improve diagnostic capabilities beyond that associated with scoring plaque accumulations and measuring pocket depths with a pocket probe, so as to identify which individuals are "infected," and to focus treatment only on those individuals. Thus, the fundamental question in regard to periodontal pathology is whether the host is responding to the nonspecific overgrowth of bacteria on the tooth surfaces (inflammatory disease) or to the overgrowth of a limited number of species which produce biologically active molecules that are particularly proinflammatory or antigenic (infection).

ARE WE DEALING WITH A DISEASE?

The question of whether we are dealing with a disease should be answered by looking at how the host responds to the dental plaque. The dental plaque is unlike any other bacterial ecosystem that survives on the body surfaces, in that it develops on the nonshedding tooth surface and can form complex bacterial communities that may harbor over 400 distinct species and contain over 10^8 bacteria per mg (200). The plaque is divided into two distinct types based on the relationship of the plaque to the gingival margin, i.e., supragingival plaque and subgingival plaque. The supragingival plaque is dominated by facultative *Streptococcus* and *Actinomyces* species, whereas the subgingival plaque harbors an anaerobic gram-negative flora dominated by uncultivable spirochetal species (44, 166). It is this gram-negative flora that has been associated with periodontal disease. Since many of its members derive some of their nutrients from the gingival crevicular fluid, a tissue transudate (35, 50) that seeps into the periodontal area, it is possible that their overgrowth is a result of the inflammatory process (146, 166).

Therefore, there is a distinction between the way the host responds to the supragingival plaque and its response to the subgingival plaque. The response to the supragingival plaque has been thoroughly studied in the experimental gingivitis model described below, whereas the response to the subgingival plaque remains under investigation. Does the host respond to the subgingival plaque as if it were an overgrowth of a bacterial community in which many members produce substances, such as LPS, that are particularly bioactive if they enter the approximating gingival tissue? Or does it respond to a plaque in which certain members produce more biologically active molecules, such as butyric acid (209) or hydrogen sulfide (233, 244), per cell or possess unique proteases, such as are found in *P. gingivalis* and *Treponema denticola*, which can degrade host molecules, creating a proinflammatory effect (84, 122, 144, 181, 304)? In either case, although bacteria are involved, it is not the scenario of a typical infection, as the offending bacteria generally remain outside the body, attached to the tooth.

Experimental Gingivitis Model

Since bacteria are always present on the nonrenewing tooth surface, even healthy gingival tissue exhibits some inflammatory cells (258). These inflammatory cells increase in number as the bacterial plaque accumulates, and the tissue becomes edematous, reddens, and eventually bleeds. The initial sequence of events in the tissue adjacent to the newly forming plaque has been extensively documented through the use of an experimental gingivitis model in which volunteers are brought to an excellent level of gingival health through repeated cleanings. They then refrain from all oral hygiene procedures for a 3- to 4-week period, after which health is restored by resuming oral hygiene and dental cleanings (146). There is a highly reproducible relationship between plaque accumulation and gingivitis, which has been interpreted as proving that the plaque mass causes gingival inflammation (144).

The initial bacterial colonizers are predominantly streptococci (290), but within days, the bacterial community changes to one characterized by *Actinomyces* species and other bacterial types (138, 290, 300). Three-week-old plaque harbors a diverse flora, as evidenced by the isolation of 166 species from only four subjects and 96 plaque samples (198). As the plaque community ages, anaerobic species such as spirochetes are detected, but in small numbers (197, 198, 300), probably indicating that the oxidation-reduction potential of the plaque has decreased to levels where microaerophilic and moderately anaerobic species can ascend to numerical prominence in the plaque (155).

If the plaque mass is held constant, only certain plaques are

associated with gingivitis, suggesting that it is the specific bacterial composition of the plaques, and not the bacterial numbers, which causes the gingivitis (161). At the time bleeding is noted, there is a significant proportional increase of *Actinomyces viscosus* and the appearance of *Campylobacter* and *Prevotella* species in the plaque. These latter species have nutritional requirements derived from the host, such as hemin and menadione, and from other microbes, such as formate. The emergence of these species at the time that bleeding is present suggests that these nutrients became available as a result of the tissue inflammation and bleeding. This proportional rearrangement of the flora, with a shift to gram-negative anaerobic species, portends the type of plaque flora which dominates in periodontal disease.

The experimental gingivitis model is stopped for ethical reasons after 3 to 4 weeks of no oral hygiene, so as to prevent any deterioration of the subject's health towards developing a more anaerobic plaque flora similar to that found in the gingivitis associated with poor oral hygiene. This "neglect" gingivitis is the most common form of periodontal disease and is experienced by all individuals at some time. It is this gingivitis that progresses to periodontitis, usually about the molars (217), where it is more difficult to maintain oral hygiene. This transition from gingivitis to periodontitis is undocumented in humans, and the trigger for the conversion is not known. In animal models this trigger for conversion is rapid plaque accumulation after placement of a foreign body such as a silk ligature around the teeth in either dogs (130) or monkeys (257). An analogous situation in humans may occur when fibrous food is retained between the teeth or when a dental restoration is poorly contoured, creating an overhang where bacteria can accumulate at the junction of the filling and the tooth surface. When amalgam restorations were purposely placed with overhangs, the numbers of both spirochetes and black-pigmented *Prevotella* and *Porphyromonas* species increased in the adjacent plaque and gingival bleeding was observed (125).

Host Response

The host mounts an inflammatory response in the approximating gingival tissue to bacterial accumulations on the teeth. This response prevents bacterial growth in the tissue; removes bacterial products such as antigens, LPS, and enzymes that have penetrated the tissue; and is associated with specific antibody formation that, in the case of *Actinobacillus actinomycetemcomitans* in the rare clinical condition known as localized juvenile periodontitis (LJP), appears to be protective (39, 60). However, the inflammatory response can also activate the matrix metalloproteases, which are the agents responsible for collagen loss in the tissues (288, 305). These latent collagenolytic enzymes can be converted to active forms by proteases and reactive oxygen species in the inflammatory environment (288), giving rise to elevated levels of interstitial collagenase in the inflamed gingival tissue (126, 305, 306). The resulting attachment loss deepens the sulcus, or depression, formed where the gingival tissues contact the tooth surface, thereby creating the periodontal pocket. By definition, this loss of attachment converts gingivitis to periodontitis.

The depth of the pocket reflects an inflammatory response that causes both the swelling of the gingival tissues at the top of the pocket and the loss of collagen attachment of the tooth to the alveolar bone at the bottom of the pocket. Good oral hygiene can reduce the inflammatory swelling (121, 134), but the attachment loss and accompanying bone loss is thought to be irreversible. Pockets tend to be stable in their depths, but some continue to extend toward the bottom of the tooth in either an intermittent (80) or gradual (113) fashion. As the pockets deepen, they provide a microbial niche where as many as 10^8 to 10^9 bacterial cells can accumulate (281). The deeper the pocket, the more isolated and inaccessible to oral hygiene procedures the subgingival plaque community becomes (321), so that these numbers remain relatively constant, with the slow microbial growth counterbalanced by the flushing of loosely adherent and dead organisms from the pocket by a tissue transudate called the gingival crevicular fluid (GCF) (35, 50).

The continuing presence of such large numbers of bacteria probably accounts for the varied host defense mechanisms against bacterial invasion and growth that can be found in the gingival tissues, i.e., high blood flow due to the presence of two separate arterial networks (258), large numbers of neutrophils in the GCF (24), large numbers of mononuclear cells in the epithelium (258), elevated immunoglobulin G (IgG) and IgA titers to specific bacterial species (61–63, 299), the formation of tissue defensins (319), and a high turnover rate of the gingival epithelium (258). This bacterial load requires additional defense mechanisms, one of which is the encasement of bacteria in hardened deposits known as dental calculus or tartar. Such deposits have been significantly associated with and have been suspected of contributing to periodontal disease (10, 46). However, subgingival calculus could be a consequence of the pocket, forming when bacterial cells at the base of the plaque die and then calcify (182). As such, calculus could be viewed as an effort by the host to prevent biologically active molecules like LPS from entering the gingival tissues, leaving only those microbes on its surface to provoke the approximating soft tissue. This interpretation would be supported by the observation in the 1988 to 1991 National Health and Nutrition Evaluation Survey (NHANES III) that 88% of sites with subgingival calculus did not bleed when gently touched with a dental instrument (37, 38, 217).

Another effective host defense mechanism is the highly vascularized gingival tissue, which presents an oxidative barrier to the penetration of the anaerobic flora from the dental plaque. The pO_2 of a 6-mm-deep pocket is about 13 to 15 -mm Hg (155), so that when bacteria living in that environment penetrate the gingival tissue and encounter a tissue pO_2 of 140 to 150 mm Hg, they are not likely to survive. While certain bacteria such as *A. actinomycetemcomitans* (47, 249), *P. gingivalis* (249), and spirochetes (243) can be detected within the tissue, they rarely are able to cause tissue necrosis. When necrosis does occur, as in noma or HIV-positive patients, the host is compromised by protein-calorie malnutrition (68) or T-cell deficiencies (204, 328). Conditions which cause a vasoconstriction of peripheral arterioles, such as smoking (30) and stress (73), are risk factors for periodontal disease, probably because the reduced blood flow allows some invading anaerobes to survive long enough in the tissues for them or their products to activate the latent interstitial collagenases. In this sense, the selection for anaerobes in the subgingival plaque may be ben-

eficial to the host, since if facultative species were dominant, tissue invasion and necrosis might be more common. For example, anaerobic species are rarely isolated from bacteremias associated with dental procedures whereas facultative streptococci and *Actinomyces* species are frequent isolates (143, 148, 245), suggesting that they can survive in the gingival tissue long enough to enter the bloodstream.

The cited defense mechanisms are overwhelmingly beneficial to the host (258), with the only long-term detriment being a slow and intermittent loss of attachment of the teeth to the alveolar bone. A small percentage of tooth sites may show a burst of 2 to 3 mm of attachment loss (80), and a small percentage of individuals may show such rapid deterioration that they lose many of their teeth at an early age. In the former case, the incidence of "active" sites is so small and the bacterial flora is so variable that it is difficult to obtain a sufficient number of patients to demonstrate unequivocal differences between active and inactive sites (59, 101, 199, 294, 320). In the latter case, these rapidly progressing forms often occur within families, raising the possibility that there is a genetic component (213). For some rare inherited and chromosomal disorders, such as Papillon-Lefevre syndrome, Ehlers-Danlos syndromes, and Chédiak-Higashi syndrome, severe early-onset forms of periodontal disease are often characteristic of the syndrome and reflect a fundamental defect in epithelial cell, connective tissue, or leukocyte function (109, 287). Such enhanced deterioration is seen in other conditions with a genetic component, such as Down syndrome (4, 16, 49) and diabetes (219, 248, 250), especially diabetes among the Pima Indians (206).

In the majority of rapidly progressing forms, the genetic component is subtle and presumably manifests as an altered host response(s) to the bacterial flora (108). In one scenario, the host monocytes would overreact to a small bacterial challenge and produce large amounts of inflammatory mediators such as prostaglandins and cytokines (214). In another scenario, defects in leukocyte mobility and/or adhesion would cause a sluggish host response in the gingival environment (314), which results in bacterial overgrowth in the plaque and their presence in the tissue. This mechanism is supported by the in vitro finding that many patients with rapidly progressing periodontitis have neutrophils and/or monocytes that exhibit various chemotactic defects (23, 218). Neutrophils taken from patients with rapidly progressing periodontitis have a significantly lower expression of L-selectin (CD62L) (176), increased basal H_2O_2 production, and decreased L-selectin shedding. The last impairment, which correlated with increased interleukin-8 levels in plasma, could contribute to initial vascular damage (72). However, even with these genetic predispositions, a trigger or bacterial challenge from the plaque flora is needed to cause the tissue loss.

ARE WE DEALING WITH AN INFECTION?

Nonspecific Plaque Hypothesis

The bacterial composition of the plaque has long defied comprehension and has led to the concept that it is the nonspecific overgrowth of any or all bacteria that causes dental disease. This tradition dates back to the 19th century, when Willoughby Miller (192), a student of Koch, had hoped to identify one or several bacterial species as being responsible for dental decay (caries). However, given the limited taxonomic data on the oral bacterial species and a complete ignorance of distinct microbial niches within the oral cavity, he concluded that caries was bacteriologically nonspecific. Miller and others reasoned that because acid demineralizes the tooth and all plaque bacteria produce acid, then all bacteria contribute to decay, especially when bacteria accumulated on tooth surfaces that were difficult to keep clean. Corrective treatment required that bacterial accumulations be eliminated and/or reduced in the retentive sites on the top of the teeth (occlusal surfaces) and between the teeth by daily toothbrushing and frequent dental cleanings or prophylaxis. Dentifrices with abrasive pumices were introduced, and the objectionable taste of the pumice was sweetened with sucrose! Mouths became cleaner, but the prevalence of dental decay approached 100% and the severity of decay resulted in tooth extractions in almost everyone, with complete tooth loss, i.e., edentulism in about 60% of older individuals (329). Dental decay became a public health problem, and only when fluoride was introduced into drinking water and dentifrices (coupled with the quiet removal of sugar) did it abate.

The treatments that were ineffective in caries control, i.e., brushing, flossing, and dental "prophylaxis," however, reduced gingivitis and became the definitive treatment modalities for the prevention of periodontal disease (217). However, an estimated 2 million to 6 million people in the United States still have periodontitis (9, 217). The treatment of this more advanced condition remained essentially the same as for prevention, namely, the debridement of the tooth at periodic intervals over a lifetime by the hygienist or dentist, supplemented, when there are deep pockets, by surgical procedures that eliminate or greatly reduce the depth of the pocket. This debridement approach is based on the premise that bacterial overgrowth per se is the cause of periodontitis.

Bacterial complexity of dental plaque. No one knows how many bacterial species, ribotypes, and serotypes coexist in the dental plaque, but the number is very large. Moore and Moore isolated 509 species from only 300 individuals, with most being previously undescribed species (200). Most cultivable species were present in such low proportions that it was difficult to associate the overgrowth of any single species with periodontal disease. With the advent of PCR technologies, many new uncultivable species are being identified. For example, when a single plaque sample was screened with a treponeme-specific oligonucleotide probe, 23 species, including 19 new species, were identified from 81 sequenced spirochetal clones (43). Although the levels of the cultivable spirochetes, *T. denticola* and *T. vincentii*, increased in plaques from diseased sites, these organisms were not the most numerous spirochetal types present in diseased sites (201).

This complexity supports the nonspecific plaque hypothesis, which contends that the overgrowth of any and all bacterial types is the trigger for the host inflammatory response. If there are more spirochetes in deep pockets than in shallow pockets (139, 166), that is because there is more space for all bacteria, including spirochetes, to grow. If there are higher proportions of spirochetes in these deep pockets than in shallow pockets, this could mean that they are preferentially selected because of

the lower pO_2 found in the deeper pockets (155). If the mean percentage of spirochetes was two to three times higher at tooth sites that bled than at sites which did not bleed, this does not mean that spirochetes are specifically associated with the bleeding; it simply means that bleeding sites are, compared to a microscopic examination of plaque, a "more time-effective and site-specific means of detecting disease" (18). The problem then is the presence of large numbers of bacteria in deep pockets, and the treatment is to clean or eliminate the pockets by surgical procedures. The bacterial findings in effect convey no useful information that would modify treatment.

Nonspecific mechanisms. Many of the proposed mechanisms by which bacteria provoke the inflammatory response are nonspecific. If the host responds in diverse ways to LPS which enters the tissue, would the LPS be more likely to come from any gram-negative species rather than from a uniquely specific organism? The prostaglandin E_2 levels observed in the GCF are highly correlated with levels of prostaglandin E_2 secreted by peripheral blood monocytes in vitro in the presence of bacterial endotoxins (216). The levels in GCF increase with the severity of the clinical condition, suggesting that the more bacterial endotoxin in the plaque, the more prostaglandin E_2 that will be secreted. Most subgingival species are proteolytic and produce an array of volatile fatty acids including butyrate, propionate, and isobutyrate (85, 208, 261), as well as sulfides such as hydrogen sulfide and methyl mercaptan, as metabolic by-products (226, 227). All these compounds could be cytotoxic to the gingival tissue, based on tissue culture studies in which propionate and butyrate inhibited the growth of cultured epithelial and endothelial cells and fibroblasts (114, 123) and in which sulfides inhibited epithelial cells (233, 244).

The question of whether these products are the result of the metabolism of the entire plaque flora rather than that of only a few species was posed by Niederman et al., who examined the relationship between the concentrations of short-chain carboxylic acids in plaque and certain putative periodontopathic bacteria with gingival inflammation in medically healthy, periodontally diseased subjects (208). After controlling for various clinical parameters, they found that gingival inflammation correlated directly and significantly with the short-chain fatty acids but not with any single bacterial species or combination of species. Subsequently, this group showed that pockets from diseased subjects exhibited a significant >10-fold increase in millimolar concentrations of butyrate and propionic acids compared to subjects with mild disease. These concentrations were significantly associated with pocket depth, attachment loss, percentage of sites bleeding when probed, and the total bacterial load in the pocket (210). These findings would implicate plaque biomass as the important contributor to periodontal pathology.

Treatment based on the nonspecific plaque hypothesis. While the above interpretations of the data are reasonable, the resulting treatment paradigm mandates that the flora be suppressed either continuously or periodically by mechanical means, so as to maintain bacterial levels compatible with gingival health. When this traditional debridement approach fails, patients are considered to have a refractory periodontitis and are suspected of either having a subtle genetic predisposition to periodontal disease or being noncompliant in their oral hygiene practices (212, 325). For these individuals, antimicrobial agents are chosen which kill as many bacterial types as possible. This encourages the usage of broad-spectrum agents such as tetracycline (110, 132) or the combination of agents such as amoxicillin and metronidazole (316). Since the plaque flora would have to be suppressed either continuously or periodically, this approach could lead to the overuse of these agents. Consider the following quote taken from a study evaluating the ability of clindamycin to control the deteriorating situation found in refractory patients: "During the year prior to entering the study, each patient had received antibiotics as part of the periodontal treatment. Tetracycline therapy ranged from one week to one year duration with most patients receiving four or five administrations of 250 mg qid for 10 to 14 days. Every patient was also treated with at least one other antibiotic and these included penicillin V, ampicillin, augmentin, erythromycin, cephalexin or metronidazole" (81). In another study, nine patients refractory to the debridement and surgical approach had been treated with either penicillin, tetracycline, minocycline, or metronidazole (309). In still another study, 17 different antimicrobial regimens were used by 23 clinicians in the treatment of recurrent (refractory) periodontitis (127).

These reports indicate that when debridement fails, clinicians do not know which antimicrobial agent to use in their search for the "magic bullet" that will kill or suppress all plaque bacteria. This is because no agent can prevent or control "all" 500-plus types of bacteria that can grow on the tooth surfaces. This is the legacy of the nonspecific plaque hypothesis, because without a targeted pathogen(s), it is very difficult to select the appropriate antimicrobial agent and design a dosage regimen that is both safe and effective. But what would be the scenario if there actually were specific bacterial pathogens in periodontal disease?

Specific Plaque Hypothesis

To change the treatment paradigm from the nonspecific reduction of plaque mass to one based on principles of antibacterial management of infections will require convincing evidence that it is the overgrowth of a limited number of bacterial species in the dental plaque that can produce the destructive inflammatory changes in the gingival tissue. Such evidence was able to change the paradigm concerning the bacterial etiology of dental decay (147).

In describing dental decay, Miller was correct in recognizing that retentive sites on the tooth surface are predisposed to decay, but he had no way of knowing that cariogenic organisms, such as the mutans streptococci and lactobacilli, are selected for in these stagnant environments. After a brief exposure to dietary sucrose, the plaque pH will quickly drop to about 5.0 to 5.5. While most supragingival plaque bacteria produce acid, they are less active at these pHs and may cease to grow. However, at this pH the tooth hydroxyapatite begins to dissolve (demineralize), serving as a buffer which allows the mutans streptococci and lactobacilli, due to their aciduricity, to survive and their numbers to increase (149). If the demineralization process is not reversed by the remineralizing potential of saliva, the mineral lost from the tooth first appears as a white spot on the tooth surface and then progresses to dental decay. Thus, the caries process reflects a selection of plaque organisms that can survive in the low-pH environment occa-

sioned by frequent access to sucrose. The nonspecific plaque hypothesis was successfully challenged when studies in germ-free animals showed that most acidogenic bacteria were not cariogenic (71) and when Keyes demonstrated the infectious and transmissible nature of dental decay in animal models (118).

If dental decay was a specific infection, why could periodontal disease not also be a specific infection resulting from the selection of bacteria that can grow in the stagnant pocket environment, using nutrients which leak into the pocket in the GCF as the result of the microbes' production of proinflammatory molecules? In the past 25 years, over 200 studies have compared the flora of disease-associated plaques with the flora found in plaques associated with periodontal health. The results have generally shown a limited number of bacterial species, mainly gram-negative anaerobes, to be significantly associated with periodontal disease (see Tables 2 to 5). These findings have not changed the prevailing treatment philosophy in periodontal disease, because of the powerful legacy of the nonspecific plaque hypothesis in dictating treatment protocols that have become the standard of care in clinical dentistry. It is difficult to change a treatment approach whose 80% level of effectiveness is accepted by the clinician (111, 189, 190) and which provides the economic infrastructure of clinical periodontology.

There are certain periodontal conditions that do not conform to the "bacterial overgrowth hypothesis," such as LJP in which the teeth are "clean," and acute necrotizing ulcerative gingivitis (ANUG), which has a sudden onset. Both of these conditions are rare, affect young individuals, and seem to involve a host component (defective neutrophils in LJP [74] and psychological stress in ANUG [51]). However, more importantly, they appear to be specific bacterial infections which can be successfully managed by antimicrobial treatments directed at specific bacterial species (58, 131). If the bacterial flora in these exceptional conditions resembles that found in the more common forms of periodontal disease, then an antimicrobial treatment approach may extend to other forms of periodontal disease.

Exceptions to the nonspecific bacterial overgrowth hypothesis. (i) Localized juvenile periodontitis. LJP occurs among teenagers, with a prevalence of about 1 to 5 in 1,000 (36, 217, 310). In its classic form, the pathology is confined to the teeth that erupt in the mouth at about 6 years of age, i.e., first molars and incisors, although deep pockets are usually not discovered until after puberty (344). These pockets are notable for small amounts of plaque and the absence of calculus, so that a "dirty mouth" could not be evoked to explain the observed attachment and bone loss. Metabolic disorders in calcium metabolism were suspected but never documented. The condition was considered degenerative and was labeled as periodontosis, and the involved teeth were often extracted (344). However, when plaques associated with these lesions were shown to contain mostly unknown species, one of which was subsequently identified as *A. actinomycetemcomitans*, a specific microbial etiology was suspected (207, 344). Traditional debridement and surgical procedures, combined with short-term usage of locally delivered antimicrobial agents and systemic tetracycline, resulted in the retention of teeth that formerly were considered hopeless (131, 186, 275). Thus, a new treatment paradigm was introduced, namely, that LJP was a treatable bacterial infection.

A. actinomycetemcomitans was well suited to assume the role of a unique periodontal pathogen, since it is not commonly found in plaque samples removed from periodontally healthy individuals. When a malachite green-bacitracin selective medium was used, *A. actinomycetemcomitans* was found only in patients with LJP and not in patients with periodontitis or gingivitis (185). When a vancomycin-bacitracin selective medium was used, *A. actinomycetemcomitans* had a significantly higher prevalence in patients with LJP than in those with periodontitis or with periodontally healthy teeth (269). It was detected using immunologic reagents in 10 to 18% of pooled plaque samples from a population seeking treatment at a dental school clinic (32, 342). This organism is acquired in early life, most probably from family members (6, 33, 91), and possesses a wide range of virulence factors including a potent leukotoxin (26, 344). *A. actinomycetemcomitans* is one of the few plaque bacteria that can invade the gingival tissues (47), and its presence results in elevated antibody titers (64, 276, 298) to its LPS antigen, its serotype-specific carbohydrate antigen (342), and to its leukotoxin (LT) antigen (60, 302, 344) in serum and GCF.

A. actinomycetemcomitans has several serotypes, and it appears that the presence of the LT determines virulence (105). Serotype b strains are most often LT^+, and significantly higher proportions of LT^+ isolates are found in diseased patients than in healthy individuals (343). Highly leukotoxic strains were found only in subjects with LJP and EOP and tended to colonize younger individuals (105). LT inhibits neutrophils within the tissues, allowing *A. actinomycetemcomitans* to persist in the tissues and even to enter the bloodstream, where it can be deposited on damaged heart valves (260) and prosthetic joints and possibly in atheromas (106). Because LT is an immunogen, the host forms neutralizing antibodies (39, 91, 302), and this may explain why the infection is limited to the first molars and incisors. In this scenario, LT is contributory to the local tissue destruction around certain teeth and antibodies to it are in turn responsible for the subsequent "immunity" of the other teeth. The presence of these anti-LT antibodies might account for the selection of LT-negative strains in older subjects and the declining prevalence of *A. actinomycetemcomitans* in plaque samples with increasing subject age (20, 246, 252).

LJP patients are thought to have a genetic defect affecting neutrophil chemotaxis (313, 337), which could explain why LJP is often seen within families (91, 340). Equally plausible would be that an *A. actinomycetemcomitans* infection is passed down between generations and between family members (6, 21). In a study of 23 families, each with a member with LJP, Gunsolley et al. (91) found *A. actinomycetemcomitans* in about 50% of the periodontally healthy subjects. This prevalence was higher than the 10 to 17% found in periodontally healthy subjects who were not related to individuals with LJP (32, 340), suggesting that transfer of this organism is likely to occur among members of a family with an LJP patient.

Patients with LJP can be treated successfully and maintained over periods of $\geq$5 years with therapy that is directed at the microbial component and that ignores the host neutrophil component (131, 186, 256, 275). The efficacy of treatment can be correlated with the ability to eliminate *A. actinomycetem-*

comitans from the plaque (48, 275, 316). This suggests that the chemotactic defect is a minor factor in treatment success and that LJP can be adequately managed as a specific bacterial infection.

(ii) Acute necrotizing ulcerative gingivitis. ANUG tends to occur in young individuals and is characterized by a sudden onset, acute pain, and necrosis of the tissue between the teeth, i.e., the interdental papilla. Vincent, in the late 19th century, described it as a fusospirochetal infection due to the prominence of these organisms in smears of material removed from the lesion. It is the trench mouth of World War I and has a long history of association with military personnel and other individuals under stress (67, 76). Bacteria, especially spirochetes and including an extremely large spirochete with more than 20 axil fibrils inserted at each end, can be found in the tissue in advance of the necrosis (136). This large spirochete has never been cultured and is seen in plaque samples associated with disease (200). Quantitative bacteriological studies, comparing diseased and healthy sites in the same patient revealed a significant increase in the number of spirochetes and *Prevotella intermedia* in the diseased sites (68, 162).

ANUG is unusual among the periodontal clinical entities in that it can be controlled by antimicrobial mouth rinses containing oxidizing agents, such as iodine or hydrogen peroxide, or, in advanced cases, by systemic agents such as penicillin. In 1962 it was reported that it could be "cured" by the short-term systemic usage of Flagyl (metronidazole) (263), and subsequently metronidazole was shown to effectively treat ANUG in a double-blind clinical trial (58). These results led to studies demonstrating that metronidazole has a unique spectrum of activity against anaerobic bacteria (293) and to its usage in medicine for anaerobic infections (70).

Is periodontal disease an anaerobic or a microaerophilic infection? If LJP and ANUG could be treated as bacterial infections, could other forms of periodontal disease also be associated with specific bacterial types and treated in the same way? The older literature identified anaerobic organisms such as spirochetes and black-pigmented *Bacteroides* species (now classified as *Porphyromonas* and *Prevotella* species), as putative periodontal pathogens (166, 175, 247). The importance of anaerobes was reinforced by microscopic examination of plaque samples which showed spirochetes increasing, both in numbers and in proportions, as the clinical condition worsened (133, 139, 164, 201, 239) and by culture studies which showed increased proportions of *Prevotella* and *Porphyromonas* species in most forms of periodontal disease (164, 270, 294). With the identification of *A. actinomycetemcomitans* as a putative periodontal pathogen, emphasis shifted from anaerobes to this microaerophilic species. Selective media were developed that allowed its detection even when it was outnumbered 1,000:1 by other plaque species (185, 269). On the basis of its prevalence in plaque samples, it was implicated as a putative periodontal pathogen in refractory periodontitis, EOP, and the rapidly progressive lesion (59, 99, 272, 294, 314).

These reports suggest that the same organisms associated with LJP and ANUG could be associated with most, if not all, forms of periodontal disease. If this is the case, then by analogy, most forms of periodontal disease could be specific, albeit chronic, infections, and their treatment should reflect this fact. But which of these patterns is the dominant one from the diagnostic perspective? Is periodontal disease an anaerobic or microaerophilic infection? In the subsequent sections, the bacteriology of EOP and adult forms of periodontitis will be discussed to see which, if any, type of bacterial specificity can be demonstrated. Because we are assigning etiological significance to the bacteriological findings, it is essential to define the clinical status of the patient and the sampled tooth site. Such caution is not needed when plaque or bacterial mass is considered as the etiological agent.

TABLE 1. Various clinical entities in periodontal disease (AAP classification schemes for periodontitis)

New classification (17)	Old classification (42)
I. Chronic periodontitis	Adult Periodontitis (AP) (adults >35 yr)
Localized	
Generalized	
Refractory	Refractory periodontitis
	Necrotizing ulcerative periodontitis
	Periodontitis associated with systemic disease
II. Aggressive periodontitis	Early-onset periodontitis (EOP) (persons ≤35 yr)
Localized	
Generalized	
Refractory	
	Prepubertal (PP)
	Localized
	Generalized
	Juvenile (JP) (puberty to about 20 yr)
	Localized (LJP) (possible neutrophil defect)
	Generalized (GJP) (systemic deficiency uncertain)
	Rapidly progressive (RPP) 20–35 yr
III. Periodontitis as a manifestation of systemic disease	
Associated with hematological disorders	
Associated with genetic disorders	
IV. Necrotizing periodontal disease	

The American Academy of Periodontology in 1999 recommended a classification scheme, which contained four primary forms of periodontitis: chronic periodontitis, aggressive periodontitis, periodontitis as a manifestation of systemic diseases, and necrotizing periodontal disease (17). Chronic and aggressive periodontitis, the two most common forms of periodontal disease, were subdivided into localized and generalized forms based on the extent of tooth involvement. The new scheme and its counterparts in the prior literature are presented in Table 1. Most categories formerly grouped as EOP are now called aggressive periodontitis, and the categories referred to as adult periodontitis are now called chronic periodontitis. Refractory periodontitis was eliminated as a separate disease category, but the refractory designation could be applied to all forms of periodontitis in the new classification scheme. All reports covered in this review used the older classification schemes, and they will be retained, although where possible they will be related to the new scheme.

Some epidemiologists have defined moderate forms of adult periodontitis as the presence of one or more teeth with a pocket of ≥4 mm in depth, with no teeth having a pocket of ≥6 mm, and advanced forms as the presence of one or more teeth with probing depths of ≥6 mm (37, 38). Others have regarded

an attachment loss of ≥3 mm about any given tooth as an indication of significant periodontal attachment loss (9, 38). The findings have been reported as (i) the prevalence of individuals with one or more teeth or tooth sites with evidence of the disease; (ii) the extent of the disease, i.e., the number or percentage of diseased teeth; and (iii) the severity of the disease in terms of the number or percentage of teeth with pocket depths or attachment loss of ≥3 mm and ≥6 mm (217).

Clinical disease is thus measured by the extent of cumulative morbidity about the teeth, and the diagnosis is based on the age of the individual. It bears little or no relationship to the inflammatory status of the adjacent gingival tissue, or the bacterial composition of the plaque. For our purposes we will classify periodontal disease as gingivitis, no attachment loss, EOP, patients under 35 years of age, and adult periodontitis (AP). Because gingivitis can be, and should be, treated by debridement procedures, we will restrict our discussion to EOP and AP to determine whether the bacteriological patterns found in LJP or ANUG are observed in either or both of these broad clinical categories. If so, the antibacterial treatment tactics used in the management of LJP and ANUG could be applied to EOP and AP.

Many of the bacteriological studies in EOP and AP are listed in Tables 2 and 3. The studies are arranged to reflect whether they were longitudinal, cross-sectional, or case studies. One would expect that data from longitudinal studies would provide the best evidence for the implication of a bacterial species in the subsequent development of periodontal pathology. The studies within each category are then stratified to reflect the method of bacterial detection and identification; i.e., studies which used DNA procedures are listed first, followed by studies which used culture, immunological, microscopic, or serum antibody titers. In some investigations, more than one method of bacterial identification was used, and they are so noted. Within each method of detection, the studies are listed according to the number of subjects or patients sampled. Thus, studies with a larger sample size are listed first, since one would suspect that data from a longitudinal study that sampled 248 subjects would have more import than a longitudinal study that sampled only 8 subjects.

The tables also show whether the study monitored for the levels of 10 bacterial species or types that have been frequently implicated as periodontal pathogens. Three microaerophilic species, i.e., *A. actinomycetemcomitans*, *Campylobacter rectus*, and *Eikenella corrodens*, are listed to the left in the column labeled Bacterial Species Monitored, followed by seven anaerobic species, i.e., *P. gingivalis*, *B. forsythus*, *T. denticola*, *P. intermedia*, *Fusobacterium nucleatum*, *Eubacterium* species, and the microscopic counts of spirochetes. If an investigator(s) monitored for the presence of only one species, it would be difficult to assess the importance of this species relative to other putative periodontal pathogens. In 5 of the 10 studies that significantly associated *A. actinomycetemcomitans* with EOP, this was the only species that was monitored, whereas when other species were monitored, *P. gingivalis*, *F. nucleatum*, and spirochetes were more likely than *A. actinomycetemcomitans* to be significantly associated with EOP (case studies not included) (Table 2). This analysis would suggest that anaerobes such as *P. gingivalis* are more likely to be associated with EOP.

At the bottom of Tables 2 and 3 is a summary of the number of studies that reported a significant result for each listed bacterial species and the number that did not report a significant finding. In some studies, where no significance was found, the authors reported tendencies, and these are noted in the Comment column. These summaries show both the inconsistency of the findings between studies and a tendency for anaerobic species to be more frequently associated with AP (Table 3) and possibly EOP (Table 2). In the discussion which follows, priority is given to longitudinal studies and to the cross-sectional studies which monitored for several bacterial types.

(i) Early-onset periodontitis (aggressive periodontitis). Our description of aggressive forms of periodontitis will include bacteriological studies on individuals 35 years or younger, with the prototype clinical example being LJP (Table 2). LJP is unique among periodontal clinical entities in that it can be unequivocally identified by its occurrence about molar and incisor teeth in young individuals in the absence of obvious plaque and calculus accumulations. However, periodontitis is also observed in other young individuals in association with plaque and calculus (7) and results in tooth loss before the age of 20 years. The prevalence of these early-onset forms varies from about 0.05 to 0.2% in European children, from 0.65 to 2.3% in U.S. children, and up to 3.7% in Brazilians (145). Some of the variability in prevalence depends on the criteria used to define disease. For the United States in 1986 to 1987, 70,000 adolescents were estimated to have LJP, another 17,000 were estimated to have a more generalized destructive form involving more teeth (called generalized juvenile periodontitis), and another 212,000 were estimated to have what was defined as an incidental loss of attachment, with one or more teeth exhibiting an attachment loss of ≥3 mm (145). In addition, there is an aggressive clinical entity seen in young adults between 18 and 35 years of age, which is known by several names, i.e., generalized EOP, generalized destructive periodontitis, severe periodontitis, or rapidly progressive periodontitis, and is estimated to affect about 1,780,000 Americans (217).

A. actinomycetemcomitans is often significantly associated with LJP (344), but much of the evidence is biased toward *A. actinomycetemcomitans* since in most studies none of the other potential periodontopathogens were screened for in the plaque samples. In a survey of 403 subjects, in which a vancomycin selective medium was used, *A. actinomycetemcomitans* was found in 28 of 29 LJP patients, in 16.9% of 142 periodontally healthy subjects, in 20.8% of 134 AP patients, and in 5% of insulin-dependent juvenile patients (342). In another example, 9 of 10 eighth-grade students who presented with an inflammatory form of EOP had *A. actinomycetemcomitans* colonizing (infecting) a mean of 14.2 teeth per child (41). In a 3-year prospective study, Ebersole et al. (60) monitored levels of *A. actinomycetemcomitans* in plaque, antibody levels to *A. actinomycetemcomitans* in serum, and loss of periodontal attachment in 28 AP patients, 11 EOP patients and 12 control subjects. All EOP patients and 16 of the AP patients had elevated serum IgG antibody titers to *A. actinomycetemcomitans*, whereas the other 12 AP patients and the controls had normal antibody levels. The patients with the elevated antibodies had more teeth colonized with *A. actinomycetemcomitans* and higher proportions of *A. actinomycetemcomitans* in their plaques than did patients

TABLE 2. Bacteriology of EOP (aggressive periodontitis)

Clinical classification[a]	Type of study[b]	No. of subjects	Method of detection[c]	Presence of bacterial species monitored[d,e]										Comment	Reference
				Micro-aerophilic			Anaerobic								
				Aa	Cr	Ec	Pg	Bf	Td	Pl	Fn	Eub. sp.	Spir		
LJP, GJP, ILA	Longitudinal (6 yr)	248	DNA	NS	NS	NS	S		S	NS	NS			Retrospective	8
LJP, EOP, AP, H	Longitudinal (≤3 yr)	51	Culture/Ab	S										Active sites	60
LJP	Longitudinal (37 days)	8	Culture-Sel	S	NS		NS			NS	NS		NS	Based on sites	183
EOP, H	Cross-sectional	272	PCR-DNA	NS										No difference H & D subjects	Becker et al., Abstract
LJP, EOP	Cross-sectional	24	DNA	NS			S			S				Pg > Pi > Aa	171
EOP, AP, H	Cross-sectional	29	DNA				S								120
At Risk	Cross-sectional	284	Culture/Micro	NS			NS			NS	NS		NS	Black-pigmented species and spirochetes	333
EOP,AP	Cross-sectional	120	Culture/Micro				S			NS	NS		S	Pg and spir in EOP, no Aa	164
LJP	Cross-sectional	105	Culture	NS										17/105 subjects with AL had Aa	309
LJP, AP	Cross-sectional	70	Culture/Micro	NS									NS	Aa in 53% of patients	203
LJP, EOP	Cross-sectional	42	Culture	NS	NS		NS	NS	NS	NS	NS	NS	NS	Fn and Eub increased	200
LJP, AP, G,H	Cross-sectional	36	Culture	S		S	NS			NS	S		S	Proportions	254
At Risk	Cross-sectional	19	Culture-Ab	NS										Aa in 9/10 EOP subjects	41
LJP, EOP	Cross-sectional	15	Culture	S	S	NS	S			S	S			Prevalence	170
EOP	Cross-sectional	15	Culture	NS	NS	NS	NS			NS	NS			Pg, Aa, and Pi sig related to AST	124
EOP/AP/H	Cross-sectional	12	Culture	NS	NS		NS			NS	NS			%Aa and Pg elevated in EOP	295
EOP	Cross-sectional	10	Culture	NS	NS	NS	S	S		NS	S	NS			116
EOP	Cross-sectional	10	Culture	NS	NS	NS	S	S		S	S	NS			117
LJP, AP, G,H	Cross-sectional	403	Culture-Sel	S											340
LJP, AP,H	Cross-sectional	43	Culture-Sel	S									S	More spir in Aa-positive plaques	274
LJP, AP,G	Cross-sectional	32	Culture-Sel	S										Prevalence	185
LJP, G	Cross-sectional	22	Culture-Sel	S											66
EOP	Cross-sectional	22	Microscopic										S	Spir in sites with P	133
LJP, EOP, AP,H	Cross-sectional	120	Ab-IFA	NS										Prevalence of Aa 66% in LJP, 49% EOP	91
LJP, EOP, AP,H	Cross-sectional	283	Serum Ab				S	NS						Most patients have Ab to Pg, not Bf	39
LJP, EOP, AP,H	Cross-sectional	242	Serum Ab	NS	NS		S			NS	S	S		Ab to Pg, Fn and Eub sig in EOP	92
LJP, EOP, AP	Cross-sectional	200	Serum Ab	S										Ab to Aa sig elevated in LJP	75
LJP, EOP, AP,H	Cross-sectional	127	Serum Ab	S	NS		NS			NS	NS			Ab to Aa sig elevated in LJP	64
LJP, EOP	Cross-sectional	72	Serum Ab	NS			NS							Aa seropos in LJP, Pg in EOP	23
EOP/Downs Sun	Case	21	DNA	NS	NS		NS	NS	NS	NS	NS			Pg, Td, Pi, Fn >> Aa	49
EOP/P-L patients	Case	12	DNA	NS	NS		NS	NS	NS	NS	NS			Td, Bf, Pi, Cr > Pg and Aa	174
LJP	Case	20	Culture/Micro	NS									NS	Aa and spir in 53% of sites	20
EOP	Case	7	Culture	NS	NS		NS			NS	NS	NS			323
EOP, RPP	Case	7	Culture	NS			NS			NS	NS			No Aa found	311
LJP	Case	6	Culture	NS			NS			NS	NS			Aa in diseased sites	268
LJP,RPP	Case	3	Culture	NS			NS			NS				Family, Aa > black-pigmented sp.	205
EOP	Case	1	Culture	NS										Black-pigmented species	262
No of studies in which significant				10	1	1	9	2	1	3	5	1	4		
No of studies in which not significant				23	12	5	14	4	3	17	13	3	5		

[a] Clinical classification: At risk, subjects who are at risk of developing periodontitis; Epi, epidemiological study; G, gingivitis; GJP, generalized juvenile periodontitis; H, periodontally healthy; ILA, incidental loss of attachment; P-L patients, Papillon-LeFevre syndrome patients; RPP, rapidly progressive periodontitis; RP, refractory patient; Tx, treated periodontal patient.

[b] Type of study: case, case study with no comparison group.

[c] Method of detection: Culture-sel, selective medium; Culture and Ab, culture and antibodies used; Ab-IFA, indirect fluorescent antibody; Cultute & micro, culture and microscopic examination; DNA, DNA probes; serum Ab, serum antibody titers measured.

[d] Bacterial species that were monitored: Aa, *A. actinomycetemcomitans*; Cr, *C. rectus*; Ec, *E. corrodens*; Pg, *P. gingivalis*; Bf, *B. forsythus*; Td, *T. denticola*; Pi/Pn, *P. intermedia/nigrenscens*; Fn, *F. nucleatum*; Eub. Sp. = *Eubacterium* species; Spir, spirochetes; Tv, *T. vincentii*.

[e] S, significant, NS, not significant.

TABLE 3. Bacteriology of AP (chronic periodontitis)[a]

Clinical classification	No. of subjects	Method of detection	Presence of bacterial species of monitored										Comment	Reference
			Micro-aerophic			Anaerobic								
			Aa	Cr	Ec	Pg	Bf	Td	PI	Fn	Eub. sp.	Spir		
Longitudinal studies														
AP	60	DNA	NS			S			S				Active vs inactive sites	168
Ap,G,H	35	DNA	NS	NS	NS	NS	NS		NS	NS			Bf and Cr increase in active sites	180
AP	16	DNA	NS	S	NS	S			NS	NS				12
AP,G,	48	Culture/DNA	NS	S		NS	S	NS	NS	NS	NS		Sn is *Selenomoas noxia*	294
AP/HIV	43	Culture/DNA	NS	NS		S	NS		NS				Pg significantly higher in HIV^+ persons	53
AP	98	Culture,Ab-E	NS			NS			NS				Aa or Pi did not predict future episodes	141
AP	38	Culture	NS	S	NS	S	NS		S	NS			*Peptostreptococcus micros* Sig	101
AP	33	Culture	NS	NS	NS	NS	NS		NS	NS	NS		site likely to be active if Bf, Pg, Pi, Cr and Aa present	59
AP	33	Culture	NS	NS	NS	NS	NS		NS	NS	NS		Fn,Bl,Cr,Pg and Pl in most disease sites	280
AP	30	Culture	NS			NS			NS					320
AP	20	Culture	NS	NS	NS	NS	NS		NS	NS		NS		129
AP,H	415	Ab-IFA	NS	NS		NS	S		NS	NS	S		Bl predicts bone and tooth loss	177
AP	201	MonoAb	NS		NS	S			NS	NS				330
AP,H	65	MonoAb/Micro		NS		NS		NS				S	*T. vincentii* is Sig	237
Cross-sectional studies														
AP,ECP	614	DNA/Culture					NS						Bf in 73% of AP, significant associated with Pg	172
AP,H	311	PCR-DNA				S								86
AP,Tx,H	203	DNA	NS	NS	NS	S	S	S	NS	NS	NS		Bf > Td, Pg > Sn, no Aa	97
AP,G,H/HIV	246	DNA	NS	NS	NS	S			NS				Pg significantly increased in HIV^- persons with AP	204
AP	185	DNA	NS	NS	NS	S	S	S	NS	NS	NS	NS		279
AP,G	150	PCR-DNA	S	NS	NS	S	S	S	S				Bf, Pg, Td, Pi, Pn > Aa	19
AP,G,H	149	DNA	NS	S	NS	S			S	S			Fn and Ec provide little information	135
AP	148	DNA	NS	S	NS	S	S	S	NS	NS			Bf, Td, Pg, and Cr significant with progressing sites	220
AP,EOP,H	29	DNA				S								120
AP	20	DNA	NS	NS	NS	NS		NS	NS	NS			All species more prevalent than Aa	285
Refract P	94	DNA/Culture	NS	NS	NS	NS	NS	NS	NS	NS	NS		Nonoral flora	52
AP,EOP	90	DNA/Culture	NS	NS	NS	NS	NS		NS	NS	NS		14 probes accounted for 27.8% of viable	102
AP	64	DNA/C/Ab-IFA/M	NS			S	S	S				S		158
AP	43	DNA/Culture	NS			NS	NS		NS	NS			Prevalence of Fn, Bf, Pg > Pi > Aa	13
AP,H	42	DNA/Culture	S			S	S	S	S	S			52% Aa by checkerboard DNA, but 0% with oligonucleotide DNA	14
AP	39	DNA/Culture				NS	NS						Bf in 70% and Pg in 35% of pockets ≥7 mm	296
AP	36	DNA/Culture	NS			NS	NS		NS	NS			prevalence of Pg > Pi > Fn > Aa	15
AP,EOP,GH	300	Culture/Micro	NS	S	NS	S			S	S	S	S	*Eubacterium* and Fn important	200
AP,H	284	Culture/micro	NS		NS	NS			NS	NS		NS	Low prevalence of Aa	333
AP,EOP	120	Culture/micro	NS			S			NS	NS		S	Pg and spir in EOP, no Aa	164
FP	196	Culture/Ab-IFA	NS			S	S			S		S	Bf most numerous	140
AP	22	Culture/micro/Ab	NS	NS	NS	NS			NS	NS		NS		283
AP	171	Culture	NS	NS	NS	NS			NS	NS			Pg, Aa, and Pi > 5%	222
AP,EOP	61	Culture	NS	NS	NS	NS			NS	NS			Fn, Ec, and Si elevated in patterns of wide disease; Aa in local	98
AP,G,H	106	MonoAb		NS	NS		NS	NS			NS		Pg found in 5% of H, 10% of G, 12% of AP	240
AP,G	76	MonoAb						NS				S	Spirochetes and Tv with AP	239
APH	42	MonoAb						NS					Ts is *T. socranskii*	238
Epi	1,361	Ab-IFA	NS	NS		S	S		NS	NS	NS		Odds ratio Bf = 2.52, Pg = 1.73	87
Epi	1,361	Ab-IFA	NS	NS		S	S		NS	NS	NS		Bf and Pg increase in smokers	341
AP,H	938	Ab-IFA	S		S	S			S	S			Pg, Pi >Aa, Fn, Ec sig associated with >5-mm pockets	331
AP	12	Ab-1FA	NS			S	S		S					45
AP,H	624	Microscopic										S		332
AP	149	Microscopic										S		285
AP,G,H	60	Microscopic										S		18
AP,H	54	Microscopic										S		215
Clinic patients	41	Microscopic										NS		178
No. of studies in which significant			3	6	1	21	12	5	8	5	2	10		
No. of studies in which not significant			34	20	22	19	13	7	27	24	10	5		

[a] For definitions, see footnotes to Table 2.

with normal antibody levels. The antibody titers, as well as the levels of *A. actinomycetemcomitans* in certain plaques, significantly increased 2 to 6 months prior to attachment loss at that site.

These reports (Table 2) provide evidence that *A. actinomycetemcomitans* infections can be significantly associated with progressive loss of attachment in certain EOP and AP patients and that the antibody response to this organism coincides with increased levels of *A. actinomycetemcomitans* in the plaque. However, because in most cases no other periodontopathic species was sought, it is not possible to infer that other species were not involved in the observed pathology. The reality is that the other periodontal pathogens not only are present but almost always outnumber *A. actinomycetemcomitans*.

In one of the first studies implicating *A. actinomycetemcomitans* in LJP, the 12 plaques with *A. actinomycetemcomitans* had an average of 28% spirochetes whereas the spirochetes averaged 2% in the 100 plaques in which *A. actinomycetemcomitans* could not be detected (274). In a cross-sectional study comparing the bacterial flora associated with various degrees of periodontal inflammation, the four sites in the two EOP patients averaged 14% *A. actinomycetemcomitans* but 32% *P. gingivalis* (294). *A. actinomycetemcomitans* was found in 47 of 533 subgingival sites (8.8%) in a cross-sectional study involving 284 patients ranging in age from 20 to 40 years (333). In 19 of these sites, *A. actinomycetemcomitans* made up, on average, less than 0.04% of the flora, and in only 6 sites did it comprise ≥1% of the cultivable flora. There was a tendency for increased pocket depth and attachment loss in the *A. actinomycetemcomitans*-positive sites compared to the *A. actinomycetemcomitans*-negative sites. In *A. actinomycetemcomitans*-positive sites associated with pockets of >3 mm, there were significantly higher proportions of black-pigmented *Prevotella* and *Porphyromonas* species and a tendency for higher spirochete counts. We examined over 400 subgingival plaques in 116 patients representing various clinical conditions and found spirochetes and black-pigmented species to be ubiquitous in EOP and AP. In 23 EOP and 4 LJP patients, spirochetes averaged 27% of the microscopic count and *A. actinomycetemcomitans* could not be detected (164).

In another study, *A. actinomycetemcomitans* was found in only 2 of 10 EOP patients whereas *P. gingivalis* was present in 92% of the plaques and accounted for 26.7% of the cultivable flora and *B. forsythus* was found in 53% of the plaques and accounted for 23.6% of the cultivable flora (116). Han et al. (104) could not associate *A. actinomycetemcomitans* with EOP among Chinese teenagers. Kuru et al. (124), in a study involving 15 EOP patients, isolated *P. gingivalis*, *P. intermedia*, and *A. actinomycetemcomitans* from 93, 80, and 50% of inflamed sites, respectively, that also were positive for the presence of aspartate aminotransferase in the gingival crevicular fluid. Aspartate aminotransferase is released when there is cell damage and correlates with gingival inflammation (225). *A. actinomycetemcomitans* was detected by PCR in pooled plaque samples in 29% of 162 periodontally healthy subjects and in 27% of 109 periodontally diseased subjects (M. R. Becker, A. L. Griffen, S. R. Lyons, and K. R. Hazard, Abstract, J. Dent. Res. Spec. Issue **985:**229, 1998). None of these reports implicate *A. actinomycetemcomitans* in EOP.

Commercially available DNA probes to *A. actinomycetemcomitans*, *P. gingivalis*, *B. forsythus*, *T. denticola*, and other putative pathogens have enabled investigators to seek these organisms in plaques removed from patients who have a genetic predisposition to EOP. In one study, 60 Down syndrome children aged 2 to 13 years were significantly more likely to be colonized by *B. forsythus*, *T. denticola*, *P. gingivalis*, *P. nigrescens*, and *C. rectus* than were age-matched children (16). In another study (49), the plaque flora of 10 Down syndrome subjects had high proportions of *P. intermedia*, *T. denticola*, *F. nucleatum*, and *P. gingivalis* and lower proportions of *E. corrodens, C. rectus*, and *B. forsythus*, whereas *A. actinomycetemcomitans* could be detected in only one patient. This same pattern, showing dominance of anaerobic species in plaque samples associated with disease, was also found in 11 cerebral palsy patients. Institutionalized Down syndrome and cerebral palsy children have high proportions of plaques capable of hydrolyzing the synthetic trypsin substrate benzoyl-DL-arginine naphthylamide (BANA) (69). Three anaerobic bacteria, *P. gingivalis*, *T. denticola*, and *B. forsythus*, are among the BANA-positive species found in plaques (150). All 12 Saudi Arabian adolescents with Papillon-Lefevre syndrome harbored four or more of the putative periodontal pathogens, with *B. forsythus*, *T. denticola*, *P. intermedia*, and *C. rectus* being present at levels of $\geq 10^6$ cells in more than half of the patients. *A. actinomycetemcomitans* and *P. gingivalis* were found at high levels in only one subject (174). These findings indicate that even when there are genetic or congenital defects that predispose individuals to EOP, the same anaerobic flora found in EOP and AP can be associated with the periodontal lesion. *A. actinomycetemcomitans* is occasionally detected in these subjects but never at levels indicating that it is contributing to the clinical pathology.

The previous studies were performed on convenience samples (i.e., the subjects were recruited without using statistical sampling techniques), so that the findings cannot be extrapolated to a general population. There are two studies in which epidemiological principles were used to relate the bacteriological findings to a wider population of young individuals. Van der Velden et al. (310) examined the periodontal condition of all school children in Amsterdam during their last year of compulsory education, i.e., 4,565 subjects with an average age of 15.8 years. A total of 230 subjects (5%) had attachment loss, and 105 of them volunteered to participate in a follow-up bacteriological study. *A. actinomycetemcomitans* was the only species sought and was found in 17 adolescents with EOP. Such a low prevalence would indicate that *A. actinomycetemcomitans* is not an etiological agent in EOP.

Albandar et al. (8) used DNA probes to assess the relationship between the plaque flora and EOP in a population of 248 U.S. adolescents. The subjects were reexamined after 6 years as part of the National Institute of Dental and Craniofacial Research (NIDR) study on the incidence of EOP in representative U.S. adolescents. These individuals were representative of 14,013 pupils in grades 8 to 12 who were examined in the 1986 to 1987 NIDR national survey of the oral health of U.S. children. The subjects were chosen to reflect a group that had attachment loss of ≥3 mm on two or more teeth and a group that had no teeth with attachment loss of ≥3 mm. The second group was randomly selected from the total population of 14,013 subjects after being matched to the first group as to gender, race, age, geographic location, and metropolitan sta-

TABLE 4. Relationship between levels of periodontopathic species and disease progression in EOP[a]

Bacterial species	No. of cells in individuals with:		Significance (*P*)
	Disease progression	No disease progression	
A. actinomy cetemcomitans	4,000	2,200	0.2
P. gingivalis	77,000	22,900	0.0001
T. denticola	159,900	53,200	0.0001
P. intermedia	60,900	38,100	0.03
C. rectus	23,400	12,600	0.08

[a] Adapted from reference 8 with permission of the publisher.

tus. The subjects were classified as general EOP ($n = 64$), LJP ($n = 26$), incidental EOP ($n = 58$), and periodontally healthy ($n = 100$) and also according to the rate of attachment loss which the subjects had experienced during the 6-year interval.

The plaque was collected on paper points from two sites in each subject, pooled, and examined for the presence of various periodontal pathogens by using DNA probes. The level of *P. gingivalis* was 3-fold higher in the general EOP group than in the LJP group, 5-fold higher than in the incidental EOP group, and 16-fold higher than in the periodontally healthy group (8). The level of *T. denticola* was three- to five-fold higher in the EOP group compared to the healthy control, whereas the levels of the other monitored species did not show any correlation with disease classification. The individuals showing disease progression had significantly higher levels of *P. gingivalis*, *T. denticola*, and *P. intermedia* than did the group with no progression (Table 4). There was no relationship between *A. actinomycetemcomitans* and disease progression. Sites with large numbers of *P. gingivalis*, *P. intermedia*, or *T. denticola* had the highest levels of β-glucuronidase activity (11). A previous study with AP patients had shown significant correlations between β-glucuronidase activity and the levels of *P. gingivalis*, *P. intermedia*, and spirochetes in the subgingival plaque flora (107). These findings suggest that the interactions between β-glucuronidase and the subgingival plaque are similar in EOP and AP and that in both, the host is responding to an anaerobic infection involving primarily *T. denticola*, *P. gingivalis*, and *P. intermedia*.

These longitudinal studies, plus studies summarized in Table 2, indicate that all forms of EOP, including those which occur in genetically susceptible individuals such as those with Down and Papillon-LeFevre syndrome, can be considered anaerobic infections, not microaerophilic infections due to *A. actinomycetemcomitans*. All bacteriological studies which implicated *A. actinomycetemcomitans* relied on the use of selective media which permitted the detection of *A. actinomycetemcomitans* even when it was present in small numbers, i.e., 0.001% of the cultivable flora (34). These studies, which looked only for *A. actinomycetemcomitans* and ignored the other members of the flora, reported a significant association between *A. actinomycetemcomitans* and the presence of clinical disease (66, 183, 185, 269) (Table 2).

Other cross-sectional studies indirectly measured *A. actinomycetemcomitans* by monitoring serum antibody levels to *A. actinomycetemcomitans* (63, 75, 91, 342). Two cross-sectional studies reported significance as a function of the number of sites in which *A. actinomycetemcomitans* was detected, but the numbers of other organisms, such as *P. gingivalis*, *F. nucleatum*, *P. intermedia*, *E. corrodens*, and spirochetes, were also significantly increased (170, 254). These considerations undermine the evidence that *A. actinomycetemcomitans* is an important periodontopathogen in LJP, let alone EOP. Only Van Winkelhoff et al. (317) found *A. actinomycetemcomitans* in all 40 EOP, 28 LJP, and 50 AP patients, where it accounted for 19% of the cultivable flora in the EOP patients, 16% in the LJP patients, and 7% in the AP patients. It was more prevalent than either *P. gingivalis* or *P. intermedia*. This is the most convincing evidence for an important role of *A. actinomycetemcomitans* in EOP.

(ii) Adult periodontitis (chronic periodontitis). The numerically more prominent clinical condition is the periodontitis found in adults older than 35 years, which may be the most common chronic infection among Americans (324). Bacteriological investigations of AP were hampered for many years by the labor-intensive cost of anaerobic culturing procedures, so that many early studies included very few patients and/or plaque samples. With limited numbers of samples to compare, it was difficult to recognize any meaningful pattern of bacterial specificity.

We reported on both the prominent cultivable flora and the microscopic counts of over 400 plaque samples taken from 120 patients, including successfully treated periodontal patients, as well as untreated EOP. AP, and LJP patients (164). Only the spirochetes were significantly elevated, in both absolute numbers and proportions, in plaques removed from untreated EOP, AP, and LJP patients compared to the values observed in the treated patients. *P. gingivalis* was significantly increased in patients with EOP. Facultative species, such as *Streptococcus sanguis* and *A. viscosus*, were significantly elevated in the treated patients, whereas *A. actinomycetemcomitans* could not be detected, even in the LJP patients. Subsequently, using DNA probes, polyclonal antibodies, and microscopy- and culture-based methods, we examined over 200 plaques removed from teeth that were scheduled to have periodontal surgery or extractions for periodontal reasons (158). *P. gingivalis*, *B. forsythus*, *T. denticola*, and spirochetes were present in 80 to 100% of the plaques, whereas *A. actinomycetemcomitans* could be detected in about 20 to 50%. These findings, using a variety of detection methods, indicated that the monitored anaerobic species were far more prevalent and dominant relative to *A. actinomycetemcomitans* in these teeth with deep pockets and attachment loss.

Other investigators have reported that *A. actinomycetemcomitans* could not be associated with periodontal disease. Christersson et al. (45) examined subgingival plaques taken from the mesial surface of all teeth in 12 patients with AP for *P. gingivalis*, *A. actinomycetemcomitans*, *B. forsythus*, and *P. intermedia*, using an indirect fluorescent-antibody technique. The three anaerobes were present in 44 to 54% of the plaques, while *A. actinomycetemcomitans* was found in only 11% of the plaques. Moore, Holdeman, and colleagues, in a series of cross-sectional studies, examined the representative cultivable floras of plaques removed from tooth sites in healthy subjects and subjects with gingivitis, LJP, generalized EOP, EOP, advanced destructive periodontitis, and AP (200). Over 509 species were identified, and about 50 species, including *Fusobacterium nucleatum*, various *Eubacterium* species, and spirochetal

species, could be significantly associated with the clinical conditions. *A. actinomycetemcomitans* could not be associated with any periodontal condition, and its proportion of the viable count, when detected, ranged from 0.3 to 1.4%. The *Eubacterium* species are rarely screened for in periodontal studies (Tables 2 and 3) and deserve further study.

In a cross-sectional study involving over 1,300 residents of Erie County, N.Y., *B. forsythus* and *P. gingivalis*, but not *A. actinomycetemcomitans*, were significantly associated with both attachment loss and alveolar bone loss (87, 88) (Table 4). The same immunological reagents and methods were used that had previously implicated *A. actinomycetemcomitans* in LJP (83). Multivariate statistical models which included clinical and demographic variables as well as the bacteriological variables showed age and smoking to be among the strongest predictors of AP. Smoking was also associated with increased prevalence of *B. forsythus* and *P. gingivalis* in these subjects (341). In a prospective study involving 415 of these subjects monitored for 2 to 5 years, the prevalence of *B. forsythus* and *Eubacterium saburreum* at the baseline examination was able to predict subsequent bone and/or tooth loss (177).

Socransky, Haffajee, and colleagues have developed a checkerboard DNA-DNA hybridization assay in which they use whole genomic probes to examine large numbers of plaque samples (90, 102, 282). They have examined over 13,000 plaques removed from 185 individuals for the presence of 40 bacterial species by using this technique (279). *A. actinomycetemcomitans* could not be associated with either increased pocket depth or bleeding on probing. Only *T. denticola*, *P. gingivalis*, and *B. forsythus*, the three species which are BANA positive (150), could be statistically associated with increasing pocket depth and bleeding on probing. These species, plus *Selenomonas noxia*, colonized significantly more tooth sites in 138 AP patients than in 30 periodontally healthy young individuals and 35 successfully treated elderly individuals. Subjects who had ≥5% of their tooth sites colonized by *B. forsythus* were 14.4 times more likely to be in the AP group than in either the periodontally healthy group or the treated group (97).

The accuracy of the whole-chromosome DNA probes compared to culturing of plaque samples, as the primary reference, has not been fully determined. The checkerboard technique was compared to culturing by Papapanou et al. (221) using 283 plaque samples from 70 dental-clinic patients. The sensitivity ranged from 0.17 for *A. actinomycetemcomitans* to 0.86 for *B. forsythus* and *Streptococcus sanguis*, and the specificity ranged from 0.17 for *P. intermedia* to 1.0 for *C. rectus*. These ranges indicate the typical finding with DNA probes, namely, that they do not correlate well with culture or serological data, usually being positive when the culture or serological findings are negative, i.e., many false positives (142, 158, 172, 179, 191, 195, 221, 255, 345). The panel of 18 probes developed by Papapanou showed that only *B. forsythus*, *P. gingivalis*, *T. denticola*, and *C. rectus (Wolinella recta)* were associated with periodontal disease in 148 Chinese subjects who had never received any periodontal treatment (220). Thus, panels of whole-genome DNA probes, made in two different laboratories, implicate the same anaerobic species in AP.

Ashimoto et al. (19), using a PCR technique, found the prevalence of anaerobes, such as *B. forsythus*, *P. gingivalis*, and *T. denticola* to increase 10.7-, 5-, and 3.4-fold, respectively, when plaques from diseased sites in adults were compared to plaques removed from sites of gingivitis in children. Microaerophilic species showed minimal changes; i.e., *A. actinomycetemcomitans* increased 2.1-fold, *C. rectus* showed no increase, and *E. corrodens* increased 1.2-fold. Lowenguth et al. (173), using DNA probes, found that *A. actinomycetemcomitans* was present in 5.5% of 219 sites, whereas *F. nucleatum* was present in 70.8%, *P. gingivalis* was present in 43%, and *P. intermedia* was present in 63.5% of the sites. These findings reinforce the importance of anaerobes in periodontal disease.

Riviere et al. have implicated, in both ANUG and AP, an uncultivable spirochete that was detected in plaque samples by using a monoclonal antibody made to detect *Treponema pallidum* (242). After ruling out the possibility that the uncultivable spirochete was *T. pallidum*, the new spirochete was given the acronym PROS (for "pathogen-related oral spirochete") and was found to be significantly associated with ANUG and adult periodontitis (238). In a prospective study, the prevalences of total spirochetes, PROS, *T. denticola*, *Treponema socranskii*, *C. rectus*, *E. corrodens*, and *P. gingivalis* were monitored in the subgingival plaque of each tooth present in 65 adults. At baseline, spirochetes were present in fewer than 15% of the 4,040 sites so monitored (239). After 12 months, only the spirochete morpho-group was significantly associated with the transition from health to gingivitis (236). In the 93 sites that developed periodontitis, the spirochete morpho-group and the PROS organism increased significantly (237).

Others showed with oligonucleotide probes that PROS represents a heterogeneous group of spirochetes (TRE I group) of which *T. vincentii* is the only cultivable member (44). When plaque samples were taken from diseased and healthy tooth sites in 53 patients with rapidly progressive periodontitis, the TRE I group spirochetes were detected in 100% of the plaques removed from deep pockets and in 34% of the plaques removed from shallow pockets (201). In contrast, *T. vincentii* was found in 21% of the deep pockets and in none of the shallow pockets, suggesting that the other uncultivable phylotypes of PROS may be more periodontopathic than *T. vincentii*.

Umeda et al. (307) examined plaques removed from pockets showing clinical symptoms of pain and suppuration and found spirochetes to account for 27% of the microscopic count and *P. gingivalis* and *B. forsythus* to account for 26 and 11%, respectively, of the cultivable count. *A. actinomycetemcomitans* accounted for only 0.3% of the cultivable count and tended to increase in proportion as a result of treatment. In a clinical trial involving the use of azithromycin in the treatment of AP patients, *P. gingivalis* was present in 9 of 44 patients, *P. intermedia* was present in 21 of 44, any black-pigmented anaerobes were present in 39 of 44, spirochetes were present in 40 of 44, and *A. actinomycetemcomitans* was present in 6 of 46 subjects (259). Still others, while monitoring the effect of locally delivered antimicrobials on the plaque flora, have noted that anaerobes are both more prevalent and more numerous than *A. actinomycetemcomitans* (31, 78, 115, 301).

In the periodontitis seen in HIV infections, the prevalences (measured by DNA probes) of *P. gingivalis* and *P. intermedia*, as well as the microaerophilic species *A. actinomycetemcomitans*, *E. corrodens*, and *C. rectus* were similar to their prevalences in plaques removed from sites of periodontitis in

HIV-negative heterosexual men. What was different was the increased prevalence of these species in plaques of both HIV-positive and -negative homosexual men compared to HIV-negative heterosexual men. The prevalence of these species was significantly higher in plaques removed from sites of gingivitis in the HIV-positive homosexual men than in those removed from gingivitis sites in the HIV-negative heterosexual men (204). Cross et al. (53) used culturing, followed by a colony lift method and DNA probes, to compare plaques from HIV-positive and -negative periodontal patients. *A. actinomycetemcomitans*, *C rectus*, *Veillonella parvula*, *Capnocytophaga ochracea*, *P. gingivalis*, *P. intermedia*, and *B. forsythus* were highly prevalent in plaques removed from both groups, i.e., 75 to 100% prevalence. The only difference between groups was a slight but significantly higher proportion of *P. gingivalis* in the HIV-positive subjects, which was attributed to a subgroup of HIV-positive subjects with widespread attachment loss. These findings and others (82, 231, 339) indicate that there is no unique plaque flora which accounts for the aggressive nature of periodontal disease in HIV-positive subjects. Rather, it is the same periodontal flora seen in EOP and AP that causes a more aggressive disease in a compromised host, much as was found in the Down syndrome and Papillon-Lefevre syndrome subjects (16, 49, 69, 174, 229).

There is the possibility that uncultivable organisms, especially the large spirochete observed in tissue sections (136) and the smaller spirochetes, such as PROS (201, 241), could be involved in the etiology of AP and EOP. While *T. denticola* is associated with both EOP and AP (Tables 2 and 4), it may not be the most important of the spirochetal species. When group- or phylotype-specific oligonucleotide probes were made to determine the frequency of known and novel treponemes in subgingival plaque samples from 53 patients with rapidly progressing periodontitis, spirochetal species other than *T. denticola* were more frequently encountered in deep pockets than in control pockets in the same patients (201). Thus, while *T. denticola* was present in 62% of the deep pockets compared to 4.5% of the control pockets, members of the TRE I and TRE IV groups were present in 100% of the deep pockets and 34 to 40% of the control sites, raising the possibility that the actual periodontal pathogens have yet to be identified.

(iii) Summary. It is difficult to compare results between laboratories when different methods were used and when the detection procedures either are unable to reliably isolate important species such as *B. forsythus* and *T. denticola* or are still in their developmental stages or are not standardized, i.e., DNA probe technology and immunological reagents. In the EOP studies, *A. actinomycetemcomitans* and *P. gingivalis* were found to be significant in about 30 and about 40% of the studies, respectively (Table 2). In the 20 studies in which both species were sought, *P. gingivalis* was found to be significant in 6 of these studies and *A. actinomycetemcomitans* was significant in 3 (Table 2). Spirochetes were significantly elevated in four of the nine studies, whereas *B. forsythus* and *T. denticola* were infrequently monitored. In studies looking at older patients (AP patients [Table 4]) *P. gingivalis*, *B. forsythus*, *T. denticola*, spirochetes, and *A. actinomycetemcomitans* were significantly increased in 52, 48, 42, 67, and 8% of the studies, respectively. Other species such as the PROS spirochete, *Eubacterium* sp., *Peptostreptococcus micros*, and *S. noxia* were occasionally associated with clinical disease (Table 4).

There does not appear to one single bacterial species that is uniquely involved in periodontal disease. Rather, periodontal disease seems to be a polymicrobial infection involving several organisms, either in combination or sequentially. If this is the case, it is unlikely that a vaccine to only one species will be effective or that a polyvaccine to all the periodontopathogens will be developed. If antimicrobial treatments are to be added to the traditional mechanical debridement procedures, the spectrum, of the antimicrobial agents and antibiotics will be important. One approach in designing a treatment protocol is to determine whether periodontal disease can be treated as an anaerobic infection, a microaerophilic infection, or a mixed microaerophilic-anaerobic infection (147). The findings shown in Tables 2 through 4, involving large numbers of samples and using diverse methods, indicate that anaerobes, rather than *A. actinomycetemcomitans* or other microaerophilic species, are more likely to be present or to dominate in plaques associated with EOP and AP. This would suggest that treatment strategies and tactics should be designed to selectively target certain anaerobic members of the plaque flora.

TREATMENT

The demonstration that anaerobic species are statistically associated with periodontal disease allows the following hypothesis to be tested: treatments that reduce the levels of or eliminate specific anaerobes such as *T. denticola*, *P. gingivalis*, *B. forsythus* and the PROS organisms, among others, from the plaque samples should result in clinical improvements.

Studies showing the effect of various treatment modalities are listed in Table 5. The conventions used in Tables 2 and 3 are repeated in Table 5, with some additions. The types of treatment are listed with the traditional debridement procedures, i.e., scaling and root planing, and surgery listed first, followed by studies using various antimicrobial regimens. The column Study Type shows whether the study was an open one in which the patient and the clinician knew which treatment was rendered or whether it was double blind or single blind. The Comment column notes whether the treatment had any significant clinical outcome. The number of studies in which a significant or nonsignificant result in the monitored bacterial flora was found is shown at the bottom of the table. Several studies did not have enough patients in the treatment groups to show significance; i.e., they were not sufficiently powered to show small differences as significant. Some authors commented on whether the treatment showed a tendency for a decrease in the monitored flora. In this regard, 9 of 12 studies showed a nonsignificant reduction in the levels, prevalence, and/or proportions of *P. gingivalis* species, which, when combined with the 15 studies that showed a statistically significant reduction, would indicate that successful treatment reduced the levels of this anaerobe.

Debridement

Treatment under the nonspecific plaque hypothesis simply involved removing the plaque from the teeth by mechanical debridement, which would be expected to reduce the levels of any monitored species. However, the tooth surfaces cannot be

TABLE 5. Response to treatment studies[a]

Clinical classification	Type of treatment	Agent	No. of subjects	Study type[c]	Method of detection	Bacterial species monitored[d]										Comment	Reference
						Micro-aerophilic			Anaerobic								
						Aa	Cr	Ec	Pg	Bf	Td	PI/Pn	Fn	Eub.sp.	Spir		
AP	S and RP[b]		57	Open	DNA	NS	NS	NS	S	S	S	NS	NS	NS			96
AP	S and RP		32	Open	DNA	NS	NS	NS	S	S	S	NS	NS	NS			54
AP	S and RP		26	Open	DNA,PCR	NS			S	S							292
AP	S and RP		21	Open	Culture	NS			S	S		S	S			*P. micros* sig	65
AP	S and RP		17	Open	Culture	NS			S			S				Aa did not change	193
AP	S and RP		7	Open	MonoAb						S					Pg and Pi deep, Pi bleeding	266
AP	S and RP		12	Open		NS						NS					321
AP	S and RP	H_2O_2 and $NaCO_3$	20	Open	Ab-IFA/micro				S		NS				S	Pg decreased in successfully treated patients	271
ECP	Surgery		23	Open	IFA	S			S							Aa decreased after surgery	93
AP	Surgery		17	Open	DNA	NS	NS	NS	S	S		S	NS	NS		Pg and Spir significantly associated with AL	278
AP	Surgery		11	Open	DNA	NS	S		S	S		S	NS		↓	Widman flap surg	128
AP,EOP	Systemic	Metronidazole	64	DB	Culture & micro											Significant clinical outcome	284
AP	Systemic	Metronidazole and amoxicillin	46	DB	DNA	NS			S			S				Significant clinical outcome	169
AP	Systemic	zithromax	46	DB	Culture & micro	NS			S			S			S	Significant clinical outcome	259
AP,EOP	Systemic	Metronidazole	40	DB	Culture & micro				S↓			S	NS↓		S	Significant clinical outcome	163
AP,EOP	Systemic	Metronidazole	39	DB	Culture & micro	NS			↓			S			S	Significant clinical outcome	159
AP,EOP	Systemic	Metronidazole	33	DB	Culture & micro	NS						S	S		S	Significant clinical outcome	152
AP	Systemic	Augmentin	21	DB	Culture & micro	NS			NS			NS	↓			No effect	326
LJP	Systemic	Tetracycline	16	DB	Culture	NS									NS	No effect	22
LJP	Systemic	Tetracycline or metronidazole	27	SB	Culture											Metronidazole better than tetracycline	256
LJP,EOP, RP	Systemic	Metronidazole and amoxicillin	118	Open	Culture-Sel	S			↓			NS				Significant clinical outcome	317
AP	Systemic	Surgery and metronidazole	46	Open	Culture	S			NS							Significant clinical outcome	211
AP,EOP	Local	Chlorhexidine	40	Open	Culture & micro	NS			S	↓						Significant clinical outcome	230
PP	Systemic	Metronidazole	27	Open	Culture											Significant clinical outcome	327
AP	Systemic	Metronidazole	9	Open	DNA	NS	NS	NS	S	S	S	NS	NS	NS		Significant clinical outcome	103
LJP	Systemic, local and surgical	Tetracycline or doxycycline	8	Open	Culture	S	↓		↓	↓		↓	↓			Surgery and systemic doxycycline best	184
AP	Local	Tetracycline	30	SB	Cult/IFA/DNA			NS								Significant clinical outcome	334
AP	Local	Tetracycline	31	SB	DNA	NS	NS	S	S			S	S			Significant clinical outcome	173
AP	Local	Tetracycline	10	Open	Culture											Pg persists in deep pockets	194
AP	Local	Metronidazole	24	SB	Culture	S			NS			S			S	Significant clinical outcome	224
No. of studies in which decrease was significant						5	1	1	15	7	4	11	3	0	6		
No. of studies in which decrease was not significant						19	7	5	12	2	1	10	9	4	2		

[a] For definitions, see Table 2 footnotes.
[b] S and RP, scaling and root planing.
[c] DB, double blind, SB, single blind.
[d] Arrows indicate a decrease in the species.

completely "cleaned" of plaque species by these procedures, even when surgery to get access to the root surfaces is performed (318). If these residual organisms are enriched per-"'centage-wise for the periodontopathic species, then the plaque community that returns could be as proinflammatory as the plaque that had just been suppressed. This would be especially true if there is an enrichment of the plaque flora by bacteria that are left behind on the root surface or that have invaded the dentinal tubules (2). Large numbers of bacteria invade these tubules and have been shown by electron microscopic examination to repopulate the root surfaces (3). This selection for periodontopathic species as a result of debridement, as well as the difficulty in controlling a biofilm such as dental plaque, might explain the failure rate of about 20% observed in clinical practice (111, 189).

The effects of scaling and root planing on the plaque flora, as monitored with whole-genome probes to 40 plaque species, were examined in 2,900 plaques in 57 patients for 6 months (96) (Table 5). Thirty-nine patients responded to treatment, and the sites that gained ≥2 mm of attachment showed a significant decrease in the levels of *P. gingivalis*, *T. denticola*, and *B. forsythus*. This reduction in the prevalence and levels of *P. gingivalis*, *T. denticola*, and *B. forsythus* was still evident 12 months later (54). The 18 patients (32%) who did not respond to this treatment, i.e., refractory patients, harbored a heterogeneous group of species not considered to be part of the dental plaque flora, such as *Acinetobacter baumanni*, *Staphylococcus warneri*, *Gemella haemolysans*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa* (52). The presence of these organisms is unusual and may reflect prior usage of antibiotics by these patients. Other investigators have found enteric organisms and staphylococcal species in low levels in about 77% of 536 Swedish AP patients (55) and in 14% of 3,050 American AP patients (273), often in refractory patients who have been unsuccessfully treated by surgical procedures or antibiotics.

Several investigators report that refractory patients have an anaerobic flora more typical of AP and EOP. Listgarten et al. (140) found a flora more typical of subgingival plaque in 196 samples removed from sites of refractory or recurrent periodontitis, i.e., *B. forsythus* was in 84% of the samples, spirochetes were in 83%, *Fusobacterium* species were in 68%, *P. gingivalis* was in 63%, and *A. actinomycetemcomitans* was in 16%. Kamma et al. (116) found *F. nucleatum* and *P. gingivalis* in 90% of 73 plaques removed from sites with depths of >6 mm in refractory patients. *A. actinomycetemcomitans* was found in only 2 of the 10 patients and in 11% of the sites. *B. forsythus* was present in 53% of the sites and, with *P. gingivalis*, accounted for 23.6 and 26.7% of the cultivable flora. Others have also found plaques high in spirochetes and *P. gingivalis* in refractory patients (94, 163).

In patients for whom debridement of the tooth surfaces resulted in clinical improvement, there was an initial reduction in the levels of bacteria, but inevitably the bacteria return to predebridement levels. For the spirochetes, this return may take 3 to 4 months (202). This gradual return gives the gingival tissue time to heal and to reestablish its myriad defense mechanisms. However, in time, if this plaque is not frequently disrupted, the inflammatory process may begin anew and the patient's disease returns and might progress. Accordingly, the standard of care recommends that the patient return at 3- to 6-month intervals for life to have his or her teeth "cleaned."

However, if specific periodontopathic bacteria were etiologically involved in periodontal disease, the efficacy of treatment could be assessed by the disappearance of these organisms from plaque samples. The elimination of a pathogen from the host tissue is a valid clinical outcome in medicine, and such an outcome would align periodontal treatments with the established protocols of infectious disease. Simonson et al. (266) showed that improved dental health following scaling and root planing was significantly associated with a decrease in *T. denticola* antigen, as measured with a monoclonal antibody. Only seven subjects were involved, so that the study was not sufficiently powered to show a decrease of the *P. gingivalis* antigen ($P = 0.09$). Others, using DNA probes and culture, have established that *P. gingivalis* and *B. forsythus* levels are consistently reduced by scaling and root planing (Table 5). And if these organisms, such as *P. gingivalis*, persist, it is because the deep pockets do not reliably allow thorough debridement (193). When surgery is employed, these organisms are further reduced (128) (Table 5).

Systemic Antimicrobials

If debridement is successful when it significantly reduces the levels of certain anaerobes, then perhaps the clinical results could be improved by the addition of antimicrobial agents to the treatment regimen. Tetracycline was first used successfully in patients with LJP in open clinical trials; i.e., either the patient or the clinician, or both, knew which treatment was given. In these studies, tetracycline was combined with surgery (131, 184, 186) or given for 3 to 8 weeks (48, 275). While clinical improvement was noted, the treatments failed to eliminate *A. actinomycetemcomitans* from all plaque samples. In open studies, metronidazole (256) or a combination of metronidazole and ampicillin has suppressed *A. actinomycetemcomitans* in the majority of plaque samples (317).

This choice of tetracycline is based on LJP being a microaerophilic infection, which is clearly not the case, as was suggested by the success of metronidazole in these LJP patients. If anaerobes are etiologically involved in EOP and AP, then an agent specific for anaerobes should be used. This was the rationale behind the choice of metronidazole in the ANUG model, where short-term use of metronidazole can be curative (58, 162). We have conducted three double-blind studies in which all patients were given scaling and root planing and then randomly assigned to receive either placebo or metronidazole for 1 week (152, 159, 163). The patients were selected on the basis of having on average of nine or more teeth with deep pockets that would normally require surgical intervention. We then diagnosed an anaerobic infection by the presence of ≥20% spirochetes and/or a BANA-positive plaque in three of the four plaques sampled. We could diagnose an anaerobic infection in over 90% of the patients who had advanced forms of EOP or AP, as would be expected from the data summarized in Tables 2 and 3. In all three studies, the clinical outcome, i.e., attachment gain and/or reduced need for surgery, was statistically more improved in the debridement-plus-metronidazole-treated subjects than in the debridement-plus-placebo-treated subjects. The subjects who were diagnosed and

treated as having an anaerobic periodontal infection achieved a statistically better clinical result than did the subjects who received the treatment that is considered the standard of care in clinical periodontology.

Subsequently, we showed that a combination of metronidazole and doxycycline, followed if necessary by local delivery of antimicrobial agents to the pocket in an ethylcellulose film, resulted in an 80% reduction in the need for surgery and extraction (153). These results have been sustained for 5 years or more (167; unpublished data). Soder et al. (284) have also shown in a double-blind trial that patients with complete healing, as defined by the absence of inflamed sites associated with pockets of ≥5 mm, were found 5 years later only in the metronidazole-treated group. In these studies, the spirochetes and/or BANA-positive organisms, after an initial significant reduction, returned to detectable levels, indicating that the species monitored by these methods were not eliminated. However, when whole-genome DNA probes were used, metronidazole plus debridement reduced *P. gingivalis*, *T. denticola*, and *B. forsythus* in plaque to undetectable levels during and immediately after the metronidazole administration. This reduction was still evident 90 and 180 days after cessation of treatment (103). In another study, whole-genome DNA probes showed that doxycycline specifically reduced the levels of *T. denticola* in plaque for at least 1 year after administration (79).

The success of both metronidazole and doxycycline would appear to be due to their specific antibacterial action against these periodontopathic anaerobes. No evidence of metronidazole resistance among anaerobes was observed in these studies, but a transient increase in doxycycline resistance among streptococci has been noted (147).

Local-Delivery Devices

These findings, i.e., that specific bacteria are etiologically involved with periodontal disease, have presaged the potential widespread usage of systemic antibiotics for the treatment of periodontal disease, with resultant overusage and misuse. This concern, as well as the possibility of some individuals experiencing an adverse reaction to systemic agents, combined with the easy access of the dentogingival surfaces, has encouraged the fabrication of vehicles to release antimicrobial agents directly into the periodontal pocket. This is a very positive development from many approaches, not the least of which is safety due to lower whole-body doses. For example, the average tetracycline content of a single 25% tetracycline fiber (Actisite) placed into a pocket is about 8 mg/tooth (77). If 12 teeth are treated for 10 days, the total tetracycline fiber dosage is about 96 mg. However, only 25% of this dosage is released during the stay of the fiber in the pocket, giving a whole-body dosage of 24 mg. Comparable reductions would result from the usage of gels containing doxycycline (228), minocycline (83), or metronidazole (5) or films containing chlorhexidine (289). These devices appear to be as effective as systemic agents in their ability to target periodontopathic species (Table 5), and they overcome the problem of patient compliance with the use of systemic agents (147, 154). Because of their proprietary nature, they have stimulated industry support and have resulted in studies culminating in Food and Drug Administration product approval.

DIAGNOSIS OF AN ANAEROBIC INFECTION

The treatments that were effective reduced the levels of anaerobic species such as *P. gingivalis* (26 of 27 studies showed a reduction), *B. forsythus* (9 of 9 studies), *T. denticola* (4 of 5 studies), and the spirochetes (7 of 8 studies), whereas, there was a lesser effect on the microaerophilic species, i.e., *A. actinomycetemcomitans* (7 of 24 studies showed a reduction), *C. rectus* (3 of 10 studies), and *E. corrodens* (1 of 6 studies) (Table 5). These bacteriological findings indicate that most, if not all, forms of periodontal disease are anaerobic infections due to the overgrowth of a finite number of mostly indigenous gram-negative anaerobic bacteria in the plaques.

How does a clinician diagnose this anaerobic infection? The unique morphology and motility of the spirochetes would allow the use of the phase-contrast microscope or dark-field microscope to monitor for their presence in plaque samples. The phase-contrast microscope, combined with a video camera, has been used at chairside to record the architecture of the plaque microbial community, the motility of its members, and the number of white blood cells (119). The dark-field microscope (139) has been used to enumerate the spirochetes in plaques removed from the most diseased site in each quadrant. Because spirochetes can be found in most individuals (166), we diagnose an anaerobic infection when at least three of the four sampled plaques each have 20% or more spirochetes. When this convention is used, the microscope can diagnose an anaerobic infection defined as significant increases in spirochete numbers and proportions in diseased sites compared to nondiseased sites (Tables 2 and 3) and can monitor the efficacy of treatment (Table 5). The microscope cannot tell whether the spirochetes are *T. denticola* or whether other periodontopathic anaerobes are present.

The plaque samples could be sent to reference laboratories for culture, immunological, or DNA probe analysis (137, 232, 338). Culture analysis would allow the growth of only the anaerobes that survive the transport process. However, among those that grow, it would permit the determination of whether the suspected periodontopathogen is resistant to an antibiotic such as doxycycline.

DNA Probes

DNA probes are not at the stage of widespread clinical utility for several reasons. The complexity of the plaque flora, with over 500 species, makes it impractical for the developer of any probe(s) to evaluate for cross-reactions against all plaque species. For example, 14 whole-genome DNA probes were validated by hybridization with 249 strains representing 51 species (90), while commercially available oligonucleotide probes were validated against panels containing fewer species (195, 253, 255, 282). A whole-genome probe to *B. forsythus* showed no detectable reactivity with 75 strains representing 14 oral species (172). This limited evaluation means that some cross-reactions with untested plaque species probably exist, and could contribute to the large number of false-positive results found when DNA probes are compared to culture results obtained on the same plaque samples (15, 135, 142, 158, 172, 191, 195, 196, 221, 235, 251, 255, 296, 297, 315, 345; K. Backman, Letter, J. Periodontol. **66:**536–537, 1995). This finding can also be explained by the presence of nonviable cells in the plaque

sample and the method errors inherent in the dispersal, dilution, and culture of plaque samples. This would not explain the presence of false-positive results which occurred when samples came back positive for *P. gingivalis* or *B. forsythus* that had been constructed with pure cultures that did not include either of these species (315). With the serological comparisons, it is possible that the immunological reagents themselves are cross-reacting with related species.

The unique DNA sequence for each putative periodontal pathogen species has not been established, and often the sequences that are used have not been published. The genetic stability of any target sequence for most probes is not known or has not been published. Genetic diversity would be expected, as was found when a panel of five DNA probes developed to *A. actinomycetemcomitans* revealed significant genetic diversity to *A. actinomycetemcomitans* when tested on 35 human isolates and 20 primate isolates (95). An arbitrarily primed PCR identified 24 *B. forsythus* genotypes among 27 test strains (89). Thus, it should not be surprising when species-specific probes for the hypervariable region of the 16S rRNA of *A. actinomycetemcomitans* failed to detect *A. actinomycetemcomitans* in plaque samples that were *A. actinomycetemcomitans* positive when whole-genome probes were used (14).

Enzyme Assays

The periodontopathogens tend to be assacharolytic, and species such as *P. gingivalis* and *T. denticola* are notable for the diversity of their proteases (84, 122, 181, 303). An arginine hydrolase, which degrades the synthetic BANA substrate, is possessed by *T. denticola*, *P. gingivalis*, and *B. forsythus* but not by most other cultivable plaque species (150). This enzyme activity can be detected in plaque samples and is significantly associated with the presence of these three species in the plaque samples (157). A 5- to 10-min chairside test, called the BANA test (167a), can be used to diagnose an anaerobic infection involving *P. gingivalis*, *B. forsythus*, and *T. denticola* (151, 156). While the BANA test does not indicate which of the three species is present, this may not be necessary since these species coexist in the same plaques (97, 172, 265, 279) and all are anaerobes. A positive BANA assay was one of the significant predictors of whether a clinician would recommend a tooth for periodontal surgery or for extraction (165). The BANA assay is comparable to DNA probes and immunological reagents for sensitivity and specificity in detecting *P. gingivalis*, *T. denticola*, and *B. forsythus* in plaque samples (157).

In Japan, a test which measures the activity of the peptidase produced by *T. denticola*, *P. gingivalis*, and *B. forsythus* to hydrolyze *N*-carbobenzoxy-glycyl-glycy-arginyl-3,5-dibromo-4-hydroxyaniline is commercially available (112). There was a close relationship between this peptidase activity and attachment loss within a 12-month period (336). The ability of plaque samples to hydrolyze the fluorescent substrate Z-Gly-Gly-Arg-AFC was significantly correlated with the levels of *T. denticola* and *P. gingivalis* in plaque samples, as measured by a quantitative monoclonal antibody assay (223).

There are few publications relating to the development of immunological assays for the detection of periodontopathic species. One test, called Evalusite, was designed to detect *A. actinomycetemcomitans*, *P. gingivalis*, and *P. intermedia* at chairside (277), but it has not been extensively tested (1) and is no longer commercially available.

Is Dentistry Ready for a Diagnostic Test?

The few diagnostic tests that have been introduced to the marketplace have not done well. For a bacteriological diagnosis to be accepted in periodontal treatment planning, the treatment paradigm has to be changed from that of treating plaque accumulations to that of treating a specific, albeit chronic, infection. If the clinician were treating infections rather than managing a disease due to plaque overgrowth, then he or she would demand that such tests be available to assist in the optimal treatment of patients. Such a sentiment was expressed by an industry representative, who commented that "(our) challenge for the future is to persuade the dental community that monitoring periodontal pathogen levels, as well as other clinical indicators of disease, is essential to providing optimal care to the periodontitis patient" (308). However, the challenge is daunting, since today, apparently, there are no commercially available diagnostic tests for periodontal disease, nor is the prospect for such tests likely, given the dismal sales of these pioneer ventures into periodontal diagnostics.

If the standard treatment continues to be mechanical debridement with or without surgical access to "clean" deep pockets of their bacterial accumulations, then there is no need for a clinician to know whether *P. gingivalis*, *T. denticola*, *B. forsythus*, or even *A. actinomycetemcomitans* is overgrown in the plaque. He or she would continue to use the periodontal probe to measure the depth of the pocket as the only diagnostic tool, knowing that deeper pockets would have more bacteria. The current use of reference laboratories for bacteriological information comes primarily for guidance in the choice of antibiotics to use in the treatment of refractory patients who have not responded to traditional debridement procedures. This is a salvage mode operation and often reflects the determination of some patients who insist on all available treatments to keep their natural teeth. This is not the place where diagnostics need to be used. Rather, they should be employed prior to any treatment to guide the clinician in his initial choice of antimicrobial approaches to be used in the management of periodontal infections. This requires a paradigm shift of considerable magnitude, namely, from treating plaque accumulations to treating an infection (147).

CONCLUSIONS

The evidence presented in this review indicates that most, if not all, forms of periodontal disease are specific, albeit chronic, infections. Regardless of whether the host is genetically predisposed to periodontal disease, as in Papillon-LeFevre syndrome or Down syndrome, or if the host is compromised by leukocyte defects as in LJP or diabetes, or is a smoker, or has poor oral hygiene, or has simply aged, the clinical symptoms are almost always significantly associated with the overgrowth of a finite number of anaerobic species, such as *P. gingivalis*, *B. forsythus*, and *T. denticola* in the subgingival plaque. This overgrowth can be periodically suppressed by mechanical debridement over a lifetime, the current treatment paradigm, or the flora can be altered by the judicious short-term usage of antimicrobial agents targeted against the specific anaerobes.

This latter approach, while supported by several double-blind clinical studies (147), goes contrary to centuries of dental teaching which states that periodontal disease results from the overgrowth of plaque on the tooth surfaces, i.e., a "dirty mouth." The challenge lies not in proving that periodontal disease is an infection but in implementing treatment procedures based on the fact that it is an infection. The antimicrobial treatment of periodontal infections will benefit from studies suggesting that periodontal disease may be a risk factor for cardiovascular disease and stroke. If an antimicrobial approach is as effective as a surgical approach in the restoration and maintenance of a periodontally healthy dentition (147, 153), this would give a cardiac or stroke patient and his or her physician a choice in the implementation of treatment seeking to improve the patient's periodontal condition so as to reduce and/or delay future cardiovascular events.

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